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Vojo Dimitrijević (1910-1980), "A Street under Snow", 1951, oil on canvas, 655x500 mm. Courtesy of Art Gallery of Bosnia and Herzegovina, Sarajevo.

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Clinical updates on diagnosing glutensensitive enteropathy

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In the last twenty years serology for the diagnosis of coeliac disease has improved substantially. As the result of our serological studies in 1998 we proposed a gentle, low-risk, and cost effective algorithm for diagnosing various forms of gluten sensitive enteropathy, using a combination of different antibody determinations, namely IgA Endomysium antibodies (EMA), Tissue-transglutaminase antibodies (IgA anti tTG, IgG anti tTG), IgA-and IgG antigliadin-antibodies. Performing routinely serologic testing contributes to a decreased rate of endoscopic interventions and improves the quality of the patient's life.

Key words: Coeliac disease, Antibodies, Intestinal biopsy, Diagnosis.

Coeliac disease is an immune-mediated enteropathy caused by intolerance to gluten in genetically susceptible individuals. In intolerant patients, gluten ingestion induces a variety of symptoms. Classical symptoms include diarrhoea, malabsorption and weight loss while clinically occult or atypical presentations of the disease range from growth failure in children, irritable bowel syndrome, anaemia, chronic fatigue, osteoporosis, dental defects and infertility to severe psychological alterations and/or seizures.

The diagnosis of coeliac disease has traditionally depended on intestinal biopsies; nowadays the diagnosis has been extended to include an array of serological markers (1). In 2005 the European and North American Societies for Paediatric Gastroenterology, Hepatology and Nutrition released a consensus statement that children with elevated tissue transglutaminase antibodies in their plasma should be referred to a paediatric gastroenterologist for an intestinal biopsy (2). Similar recommendations for adults and children were put forward by the US National

Institute of Health (3). The conclusion resulting from above recommendations is that coeliac disease is diagnosed when the duodenal or jejunal mucosa displays a villous atrophy, crypt hyperplasia and an increase in intraepithelial lymphocytes (Marsh 3a,b,c) (1, 2, 3).

Is the importance of small bowel biopsy overrated?

In our opinion the above recommendations overestimate the importance of small bowel biopsies:

A loss of villous height is considered to be pathognomonic for gluten sensitive enteropathy by many clinicians, thus, it is important to emphasise the *nonspecific* nature of this finding and that individual differences in villous architecture across the population can be dramatic[1]

The biopsy should not be considered as the *„gold standard"* because it is not pathognomonic. Different diseases not related to gluten sensitive enteropathy, such as cow's milk intolerance, gastroenteritis, giardiasis, eosinophilic gastroenteritis, bowel ischemia, severe malnutrition, diffuse lymphoma of the small intestine, autoimmune enteropathy, hypogamaglobulinemia and peptic duodenitis can induce flat mucosa mimicking coeliac disease. Importantly, patients with gluten sensitive enteropathy and normal small bowel mucosal architecture have also been described shedding doubt on the dogma of the necessity of villous atrophy and crypt hyperplasia for diagnosis of coeliac disease (4, 5).

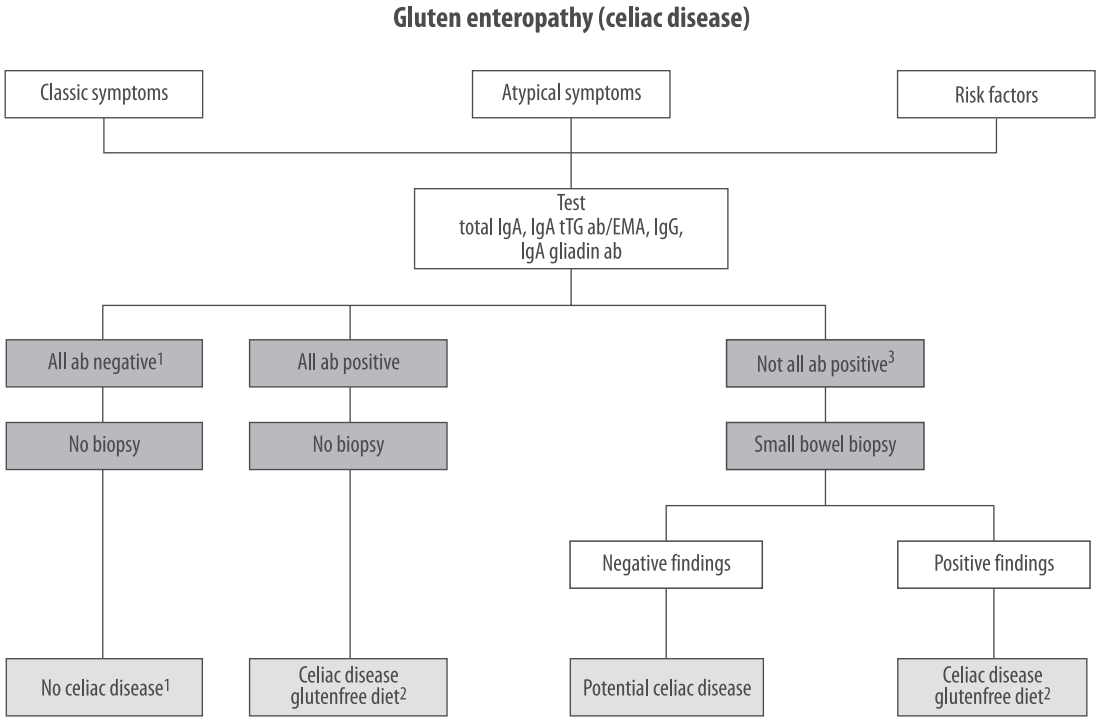
Technical insufficiency is another disadvantage of small bowel biopsy: by grasp biopsy forceps or endoscopic procedure, the biopsy specimen was considered satisfactory in only 90% of cases (6). Finally, although the *procedure* is considered to be safe, 1.5% out of 1007 biopsies haemorrhaged with 0.3% requiring transfusion and 0.3% had a laparotomy following small bowel perforation (7). Small children have to undergo general anaesthesia with a significantly higher risk for complications if compared to no risk from obtaining blood for serological diagnosis.

The role of antibodies today in diagnosing coeliac disease

As the result of our studies we proposed in 1998 a gentle, low-risk, and cost effective algorithm for diagnosing various forms of gluten sensitive enteropathy (8). The following serological tests should be performed if coeliac disease is suspected: IgA tissue transglutaminase - (IgA tTG) and IgA endomysium antibody (EMA) determination as well as IgA and IgG gliadin antibody assessment (Figure 1). In the case of IgA deficiency IgG tTG antibody determination should be performed.

In our laboratory, the sensitivity of the IgA tTG and IgA EMA antibody test is 96%. Specificity was found for IgA EMA 97% and IgA tTG of 96% (9, 10, 11) when using human tissue transglutaminse as the antigen. In other laboratories similar values are obtained depending on the quality of the test used (12). Nevertheless, the discordance of 4% between positive IgA tTG and positive IgA EMA found in our patients is high enough to advocate both tests being used simultaneously to achieve the best possible predictive value for the active disease.

More importantly Figure 1 shows that the additional determination of IgG and especially the IgA gliadin antibody increases the diagnostic significance of antibody assessment. Following a former study (9) together with recent data, the positive predictive value of positive IgA tTG/EMA and IgA and IgG gliadin ab was 99.8% (Table1). In 641 out of 642 patients pathological (Marsh 3a,b,c) mucosa was found. This constellation of antibodies has an extremely high positive predictive value and abrogates the necessity



1 Total IgA must be normal for age. If low total IgA, test IgG tTG ab, when positive → biopsy
2 Clinical remisson after a period of gliadinfree diet is additional evidence for diagnosis of celiac disease
3 If only IgG gliadin ab are present: low evidence for celiac disease, observe the patient, repeat the tests before performing a biopsy

Figure 1 Algorithm for diagnosing coeliac disease

of small bowel biopsy. However, this is not generally accepted. ESPGHAN, NSPGHAN and NIH recommend performing a biopsy in every patient with positive IgA tTG (2, 3). Since the predicting value of combined determinations is 99.8%, these recommendations induce 58% of unnecessary biopsy procedures! Furthermore, we would miss at least 6.3% of patients with coeliac disease if we depended exclusively upon one positive tTG antibody result.

In our opinion a small bowel biopsy should be performed after discordant or equivocal serological results (Figure 1). AGA and EMA/tTG may not be present at the same time in the course of the disease (9). Patients with coeliac disease and lesser degrees of villous atrophy may have equivocal or negative serological results more often. The negative predictive value of all antibodies in combination is as high as 98% (9, 10, 11). Despite the higher predictive value of positive antibodies, the majority of gastroenterologists today insist on small bowel biopsy, while the same clinicians accept that a negative antibody result does not require small bowel biopsy. This attitude is even more astonishing because patients with undiagnosed coeliac disease left on a gluten rich diet have a higher risk of malignancy and other comorbidities, while a gluten free diet in a non-coeliac patients is certainly inconvenient but harmless for the patient. Furthermore, the consensus papers do not require gliadin antibodies to be negative. If a negative tTG result is the only exclusion parameter, as recommended, than according to our population, 6% (63/1100) coeliac

patients would have been missed (Table 1). In our cohort the chance of coeliac disease (Marsh 3a,b,c) in a seronegative patient is as low as 0.08% (9/1100 coeliac patients). The group of patients with partial villous atrophy and sero-negative results who have coeliac disease is harder to identify (13, 14) and it represents a real challenge for gastroenterologists. It is our policy to observe all our sero-negative patients in regular periods in order not to miss one of the rare cases of seronegative coeliac disease which occurs mainly in adults.

Monitoring compliance to a gluten free diet

After the introduction of a gluten free diet in a patient with newly diagnosed gluten sensitive enteropathy, the decrease in plasma antibody concentration is an excellent parameter for disease follow up (10, 11). It is important to realize that it takes up to 12 months or even more on a gluten free diet for antibodies to disappear (10, 11). IgA gliadin antibodies decrease quickly and in 6-12 weeks they are no longer detectable. IgA tTG or EMA needs 10 – 12 months and IgG gliadin antibody even longer to disappear completely.

It is a reality that although aware of coeliac disease and having no problems in obtaining a gluten free diet and with adequate support from dieticians, 30% of our patients are noncompliant to strict gluten free diet! Regular monitoring of antibodies is an important tool and an excellent indicator of compliance, helping patients to keep on the gluten free diet.

In the case of diet failure a discordant rise in antibodies takes place. AGA is the first to be detected in plasma, followed by IgA tTG/EMA. If compliance failure continues over a long time, AGA disappears in most patients, while IgA tTG/EMA is detectable over many years on a continuous diet containing gluten.

Conclusion

In conclusion, it is our experience over the past 20 years that the approach now summarized in the algorithm (Figure 1) is safe, gentle and cost effective for diagnosing and monitoring coeliac disease. In view of the diverse presentation of coeliac disease, knowledge of the limitations of both serologic testing and small bowel biopsy interpretation is important. We entirely agree that it is important to avoid the self-fulfilling prophecy, taking biopsies only from IgA EMA positive individuals (15). Those patients with all positive antibodies do not need a biopsy. Performing serologic testing routinely should not result in increased biopsy interventions. In contrast if used properly it should contribute to a decreased rate of endoscopic interventions and improve the quality of the patient's life. It is time to acknowledge adequately the importance of antibody assays.

Conflict of interest: The authors declare that they have no conflict of interest. This study was not sponsored by any external organisation.

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A polymorphism in gasdermin B (*GSDMB*) gene is associated with severe asthma exacerbations in childhood: A population-based birth cohort study

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Rationale. Markers on chromosome 17q12 have been associated with childhood asthma in genome-wide association studies. **Objective.** We investigated the association of a single nucleotide polymorphism (SNP) in *GSDMB* (rs7216389) on 17q12 with asthma presence and severity in a population-based birth cohort study. **Methods.** Children were followed from birth to age 8 years. Data on parentally-reported symptoms were collected using an interviewer-administered questionnaire at age 1, 3, 5 and 8 years. Atopy was assessed by skin testing at age 3, 5 and 8 years. Information on asthma/wheeze hospital admissions and severe asthma exacerbations was collected from child's primary care medical record. Data were analyzed as a recessive genetic model, with T-allele homozygotes as the risk group. **Results.** Compared to C allele carriers, T-allele homozygotes of rs7216389 were significantly more likely to: wheeze at age 3, 5 and 8 years; have persistent wheeze (OR 1.69, 95% CI 1.05-2.71, p=0.03); have frequent episodes of wheezing and be on asthma medication. In a multiple logistic regression model adjusted for gender, atopic sensitisation and maternal smoking, T allele homozygotes were significantly more likely to be hospitalized (aOR 2.20 [1.22-3.99], p=0.0009) with Cox regression hazard ratio for T-allele homozygotes of 1.94 [1.13-3.33], p=0.016. Results of Cox regression analysis investigating the effect of genotype on the age of first severe exacerbation of asthma indicated an overall hazard ratio of severe asthma exacerbation among T-allele homozygotes of 1.53 [1.04-2.27], p=0.03. **Conclusions.** This is the first population-based birth cohort study to confirm that the risk of childhood wheeze and severe asthma/wheeze exacerbations is increased among rs7216389 TT homozygotes.

Key words: Childhood asthma, Genetics, rs7216389, GSDMB, ORMDL3.

Introduction

Twin and family studies indicate the strong genetic component of asthma (1). However, despite intensive work

ranging from family linkage and candidate gene association studies, through to genome-wide association studies (GWAS), genetic studies have produced heterogeneous results with little replication (2). Numerous genes and gene regions have been associated with asthma, e.g. Ober *et al* in their review in 2006, identified over a 100 genes which have been associated with asthma and atopy-related phenotype, of which 79 have been replicated in more than one population (2). The first GWAS on asthma published in 2007 identified a region on chromosome 17q21, where 7 of the 12 markers below 1% false discovery rate threshold mapped to a 112 kb region (3). Although several single nucleotide polymorphisms (SNPs) were associated with childhood asthma in this study, the strongest statistical association was with rs7216389 SNP in Gasdermin B (*GSDMB,* also known as *GSDML)* gene. Measurement of the global gene expression levels in lymhoblastoid cell lines revealed the same SNP (rs7216389) to be positively associated with transcript levels of orosomucoid-1 like 3 (*ORMDL3*) gene, suggesting that *ORMDL3* expression may be regulated by this *GSDMB* SNP (3). The rs7216389 SNP lies in the intronic region of *GSDMB*, approximately 100kb downstream of *ORMDL3,* which is why it has sometimes been referred to as the *ORMDL3* SNP. The roles of both *GSDMB* and *ORMDL3* genes are unknown; *GSDMB* is a member of the Gasdermin family (*Gsdm*) of genes that are expressed in skin and gastrointestinal tract epithelium (4). *Gsdm* family has been linked to gastrointestinal malignancies and a recent study suggested that *GSDMB* could play a role in stem cell proliferation (5). The *ORMDL3* belongs to a family of genes that encode transmembrane proteins of endoplasmic reticulum (ER) and is thought to be involved in protein folding (6).

Since the original GWA study (3), the association between childhood asthma and rs7216389 has been replicated in several case-control and family-based studies conducted in different populations (7-11). A case-control study investigating the association between polymorphisms in this SNP and asthma in children and young adults found that T allele was significantly more common among subjects with asthma (12). Moreover, TT genotype was a significant predictor of severe asthma exacerbations in school age children. A selected birth-cohort study of children born to asthmatic mothers extended these findings by showing the association of TT genotype with early rather than late-onset wheeze (13). We investigated the association of this common SNP in *GSDMB* (rs7216389) with asthma and wheeze within a population-based birth cohort study in which meticulous longitudinal phenotyping has been carried out from birth to age 8 years.

Methods

Study design, setting and participants

The Manchester Asthma and Allergy Study is an unselected population-based birth cohort described in detail elsewhere (14-18). Parents gave written informed consent and children their assent as appropriate, including the permission to collect data held in primary care records. The study was approved by the Local Ethics Committee and is registered as ICRCTN72673620.

Data sources

Clinical follow-up: Subjects were recruited prenatally and followed prospectively, attending clinical follow-ups at ages 1, 3, 5 and 8 years (± 4 weeks) (14-18). Information on parentally-reported symptoms and medication used at each follow-up was collected using interviewer-administered validated questionnaires. Physical examination

was carried out to confirm the presence of eczema.

Primary care data extraction: UK primary care physicians (General Practitioners-GPs) are legally required to maintain accurate records of all health-care encounters of their patients, including retention of hospital admission discharge letters, outpatient appointments and all prescriptions; this medical record follows the patient as they change GP. A trained paediatrician (ASJ) extracted all data from electronic and paper-based records on hospital admissions for wheeze/asthma, with child's age (in days) at admission.

Definition of variables
Clinical follow-up

Current wheeze: defined as a positive answer to the question "Has your child had wheezing or whistling in the chest in the last 12 months?"

Wheeze phenotypes: based on prospectively collected data, children were assigned as *No wheezing* (no wheezing ever at any follow-up by age eight), *Transient early wheezing* (wheezing only during the first three years of life), *Intermittent wheezing* (wheezing at one time point only during first five years or at age eight only), *Late-onset wheezing* (wheezing started after age three years) and *Persistent wheezing* (wheezing during the first three years, wheezing in the previous 12 months at age five and eight) (19, 20).

Atopic sensitization: ascertained by skin prick testing to common inhalant and food allergens at age 3, 5 and 8 years (21); sensitisation was defined as a mean wheal diameter 3 mm greater than negative control to at least one allergen.

Eczema: Current eczema was defined as a positive answer to the question "Has your child had an itchy rash coming and going for at least six months". Eczema was confirmed by physical examination at age 3, 5 and 8 year follow-up visits.

Current rhinitis: defined as sneezing or runny or blocked nose in the absence of cold or chest infection in the previous 12 months at age 5 and 8 years.

Primary care data extraction

Admission for asthma/wheeze in the first year of life: dichotomous outcome confirming whether the child was hospitalized for asthma/wheeze by age 12 months.

Admission for asthma/wheeze after the first year of life: dichotomous outcome confirming whether the child was hospitalized for asthma/wheeze after the 1st year of life.

Admission for asthma/wheeze in the first eight years of life: any hospital admission for asthma/wheeze in the first eight years of life.

Age at the first admission for asthma/wheeze: age of child (in days) at the first hospital admission.

Severe asthma/wheeze exacerbations: we used the American Thoracic Society definition (22) of either receipt of oral steroids for at least 3 days or admission to hospital or emergency department visit because of asthma/wheeze requiring oral steroid use.

Genotyping

SNP rs7216389 on chromosome 17q21 was selected for genotyping based on the association with childhood asthma from previous studies (3, 13).

Genomic *DNA* was extracted from blood (phenol-chlorophorm method), saliva (Oragene®-DNA Self-Collection Kit, following manufacturer's instructions) or buccal swab samples (whole Genome Amplification reaction with Illustra GenomiPhi V2 DNA Amplification kit™ [GE Healthcare], followed by purification using Illustra MicroSpin G-50 Columns™ [GE Healthcare]).

Genotyping was performed using the Single Base Extension method Sequenom® MassARRAY® iPLEX™ Gold system (Hamburg, Germany), combining a primer exten-

sion reaction chemistry with MALDI-TOF mass spectrometry (23-25). Genotyping calls were made with MassARRAY® Typer Analyser realtime software.

Statistical analysis

Data were analysed as a recessive genetic model, with T allele homozygotes as the risk group. Single time-point categorical associations were assessed using chi-squared test and logistic regression models. Longitudinal analyses were performed by Generalized Estimating Equations (GEE) using the exchangeable correlation structure and the logit link function.

We investigated the effect of genotype on the age of first severe exacerbation of asthma/wheezing resulting in admission to hospital using Cox regression and Kaplan-Meier curves. The children were retained in the analysis from birth until age at the first admission to hospital, drop-out, or age 8 years, whichever came first. SPSS 15.0 was used for all analyses. All estimates are reported with 95% confidence intervals [CI] in brackets.

Results

Participants

Genotyping success rate was 96.8%. Observed genotype frequencies did not deviate significantly from expected frequencies under the Hardy-Weinberg Equilibrium assumption and were consistent with other populations (26% TT, 48% CT, 26% CC). Of the 959 children who were successfully genotyped, 116 took part in an intervention study (26-28) and were excluded from further analysis, as were 41 non-Caucasian children. Clinical follow up data was available on 780 children at age 1, 764 at age 3 years, 778 at age 5 years and 765 at age 8 years; 696 participants had data available on hospital admissions for wheeze/asthma from the primary care records. All evaluable data is presented at each time point.

Descriptive data

Among 765 children who had data on the rs7216389 genotype and longitudinal wheeze phenotypes, 332 (43.4%) never wheezed, 194 (25.4%) had transient wheeze, 92 (12.0%) intermittent wheeze, 43 (5.6%) late-onset and 104 (13.6%) persistent wheeze. Inhaled corticosteroids were used by 91/762 (11.9%) children at age 5 and 66/767 (8.6%) at age 8 years, and inhaled bronchodilators by 152/762 (19.9%) at age 5 and 116/802 (14.5%) at age 8 years.

A total of 55/696 children (7.9%) had been hospitalized for wheeze/asthma at least once during the first 8 years of life; 38 (5.5%) were admitted once and 17 (2.4%) were admitted two or more times; 111/696 children (15.9%) had at least one severe asthma exacerbation in the first 8 years of life.

Atopy was present in 141/673 (21%) children at age 3, 197/723 (27.2%) at age 5 and 213/717 (29.7%) at age 8 years. Current rhinitis was reported by 215/769 (28.0%) children at age 5 and 212/762 (27.8%) at age 8 years.

Outcome data

Tables 1 summarizes clinical outcomes by rs7216389 in the cohort.

Current wheeze: There was no significant association between genotype and wheezing in the first year of life, whilst at ages 3, 5 and 8 we observed trends for T allele homozygotes to have more wheezing than C allele carriers. In multiple logistic regression models adjusted for gender and concurrent sensitization, T allele homozygotes were significantly more likely to have current wheezing at age 5 (adjusted OR 1.47 [1.00-2.17], p=0.05) and age 8 (aOR 1.64 [1.05-2.58], p=0.03), with a strong trend observed at age 3 years (aOR 1.47 [0.99-2.18], p=0.055).

Table 1 Respiratory clinical outcomes by rs7216389 SNP in the cohort

Outcomes	TT	CC+CT	OR (95% CI)	P value
Current wheeze age 1 year (n=780)	74/204 (36.3%)	204/576 (35.4%)	1.04 (0.74-1.44)	0.83
Current wheeze age 3 years (n=764)	58/201 (28.9%)	129/563 (22.9%)	1.36 (0.94-1.96)	0.09
Current wheeze age 5 years (n=778)	55/202 (27.2%)	121/576 (21.0%)	1.40 (0.97-2.03)	0.07
Current wheeze age 8 years (n=765)	44/202 (21.8%)	91/563 (16.2%)	1.44 (0.97-2.16)	0.07
Wheeze phenotypes (n=765)				
Never (n=332)	82/202(40.6%)	250/563 (44.4%)		
Transient (n=194)	47/202 (23.3%)	147/563 (26.1%)	0.98 (0.65-1.47)	0.9
Intermittent (n=92)	26/202 (12.9%)	66/563 (11.7%)	1.20 (0.72-2.02)	0.5
Late- onset (n=43)	10/202 (5.0%)	33/563 (5.9%)	0.92 (0.44-1.96)	0.8
Persistent (n=104)	37/202 (18.3%)	67/563 (11.9%)	1.68 (1.05-2.70)	0.03
Bronchodilator usage age 5 years (n=762)	54/198 (27.3%)	98/564 (17.4%)	1.78 (1.21-2.61)	0.003
Inhaled corticosteroid usage age 5 year (n=762)	32/198 (16.2%)	59/564 (10.5%)	1.65 (1.04-2.63)	0.03
Bronchodilators usage age 8 year (n=802)	39/209 (18.7%)	77/593 (13.0%)	1.54 (1.01-2.34)	0.045
Inhaled corticosteroid usage age 8 year (n=767)	25/203 (12.3%)	41/564 (7.3%)	1.79 (1.06-3.03)	0.03
Admission to hospital for asthma/ wheeze by age 8 years (n=696)	22/181 (12.2%)	33/515 (6.4%)	2.02 (1.15-3.57)	0.01
Admission to hospital for asthma/ wheeze in the first year of life (n=696)	10/181 (5.5%)	16/515 (3.1%)	1.82 (0.81-4.10)	0.1
Admission to hospital for asthma/ wheeze after the first year of life (n=696)	16/181 (8.8%)	19/515 (3.75)	2.53 (1.27-5.04)	0.008
Severe asthma/wheeze exacerbations by age 8 years (n=696)	38/181 (21.0%)	73/515 (14.2%)	1.61 (1.04-2.49)	0.03
Severe asthma/wheeze exacerbations in the first year of life (n=696)	10/181 (5.5%)	23/515 (4.5%)	1.25 (0.58-2.68)	0.5
Severe asthma/wheeze exacerbations after the first year of life (n=696)	34/181 (18.8%)	67/515 (13.0%)	1.55 (0.98-2.43)	0.06

TT = mutant homozygote; CC = wild type homozygote; CT = heterozygote.

In a longitudinal model (including data from ages 3, 5 and 8), we found a significant association between genotype and development of wheezing, with T allele homozygotes being significantly more likely to have wheezed throughout childhood (OR 1.36 [1.04-1.84], p=0.02).

Phenotypes of wheeze: Compared to children who had never wheezed, persistent wheezers were significantly more likely to be T allele homozygotes (OR 1.69 [1.05-2.71], p=0.03). We found no association between genotype and late-onset wheeze (p=0.8) or transient early wheeze (p=0.9).

Wheeze frequency: T allele homozygotes were significantly more likely to have had frequent episodes of wheezing in the last 12 months at age 8 years than other genotype groups (e.g. 4 or more wheezy episodes, TT 9% vs. CC+CT 4.3%, p=0.04).

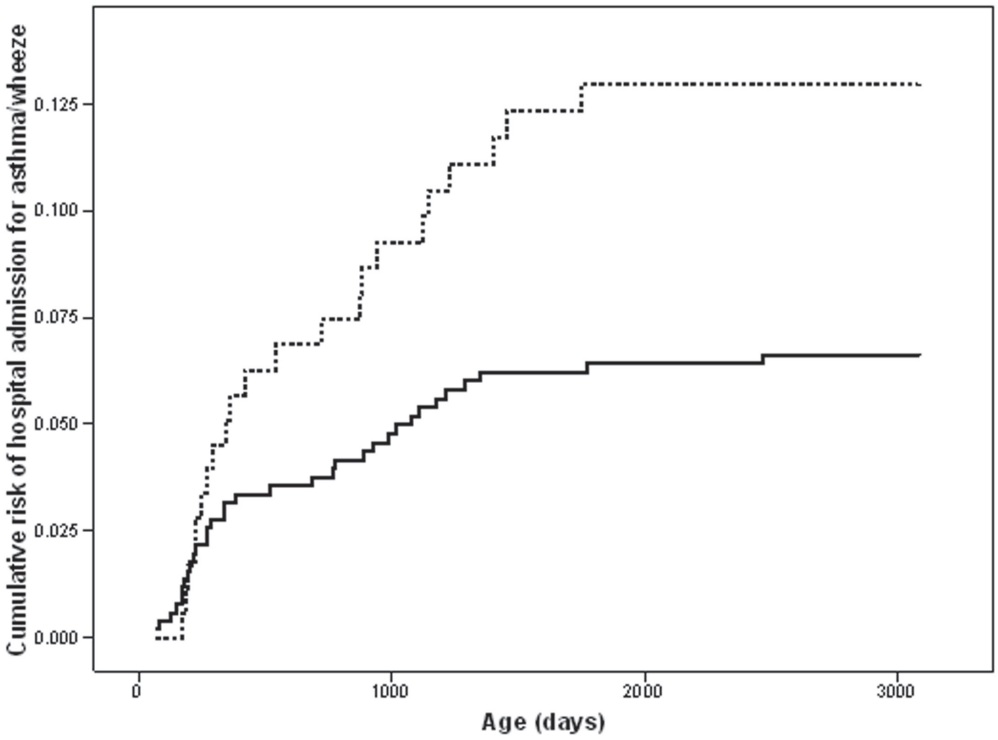

Figure 1 Kaplan-Meier Estimates of Cumulative Risk of hospital admission with wheeze/asthma in two rs7216389 genotype groups. Dashed line: TT homozygotes; Solid line: CT heterozygotes + CC homozygotes

Asthma medication: The use of both bronchodilators and inhaled corticosteroids at ages 5 and 8 years was significantly more common amongst children who were T allele homozygotes compared to C allele carriers (Table 1).

Hospital admission for asthma/wheeze

The proportion of children admitted to hospital in the first 8 years of life was significantly higher in T allele homozygotes compared to other genotype groups (12.2% vs. 6.4%, p=0.01, Table 1). In a multiple logistic regression model adjusted for gender, atopic sensitisation and maternal smoking, T allele homozygotes were significantly more likely to be hospitalized for wheeze/asthma compared to C allele carriers (aOR 2.20 [1.22-3.99], p=0.009).

In the analysis investigating the effect of genotype on the age of first hospital admission with asthma/wheeze, Kaplan-Meier plots suggested an increased risk of hospital admission amongst T allele homozygotes (Figure 1), with the results of a Cox regression analysis indicating an overall hazard ratio in this genotype group of 1.94 [1.13-3.33], p=0.016.

Further analysis amongst children with a history of wheezing revealed that the TT genotype was not associated with hospital admission in the first year of life, but increased the risk of admission beyond the first year (OR 2.34 [1.12-4.87], p=0.02, Table 2).

Severe exacerbation of asthma/wheeze

Children with TT genotype were significantly more likely to have had severe asthma exacerbation compared to those with CC and CT genotype (OR 1.61 [1.04-2.49], p=0.03). The association remained signifi-

Table 2 Admission to hospital and severe asthma/wheeze exacerbations by rs7216389 amongst children who wheezed in the first 8 years.

Outcomes	TT	CC+CT	OR (95% CI)	P value
Admission to hospital for asthma/wheeze in the first year (n=259)*	8/78 (10.3%)	14/181 (7.7%)	1.36 (0.55-3.40)	0.5
Admission to hospital for asthma/wheeze after the first year (n=259)*	16/78 (20.5%)	18/181 (9.9%)	2.34 (1.12-4.87)	0.02
Severe asthma/wheeze exacerbation in the first year of life (n=259)*	8/78 (10.3%)	18/181 (9.9%)	1.04 (0.43-2.49)	0.9
Severe asthma/wheeze Exacerbation after the first year of life (n=259)*	30/78 (38.5%)	52/181 (28.7%)	1.55 (0.89-2.71)	0.1

* Analysis conducted among children who wheezed in the first eight years of life only
TT= mutant homozygote; CC= wild type homozygote; CT= heterozygote .

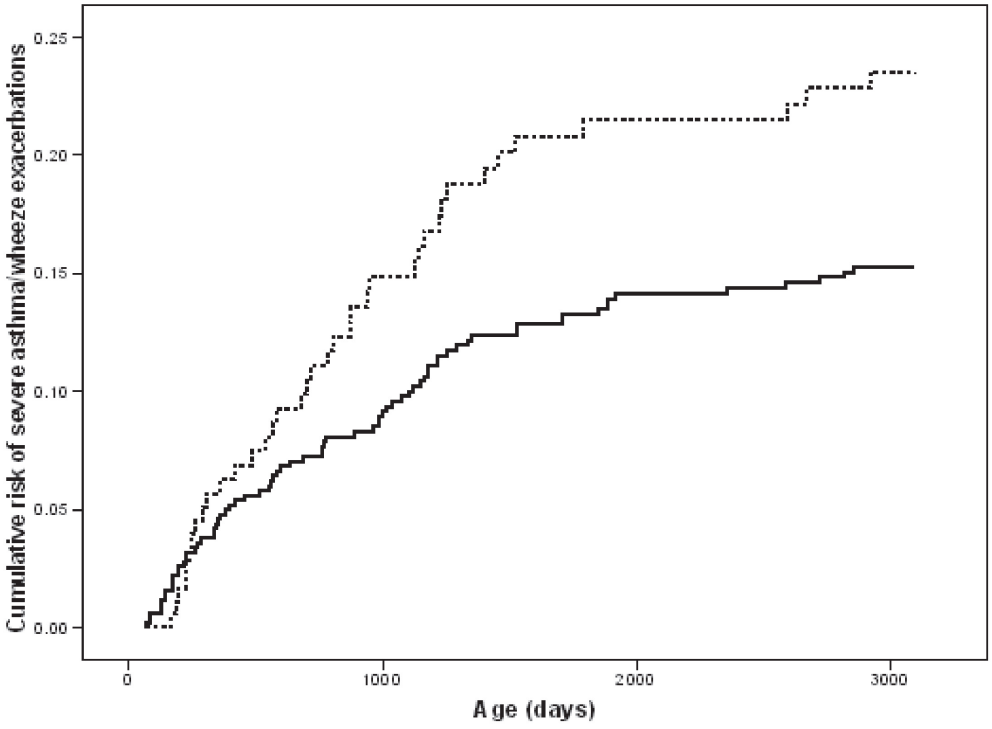

Figure 2 Kaplan-Meier Estimates of Cumulative Risk of severe asthma/wheeze exacerbations in two rs7216389 genotype groups. Dashed line: TT homozygotes; Solid line: CT heterozygotes+ CC homozygotes

cant after adjusting for gender, atopic sensitisation and maternal smoking (aOR 1.73 [1.10-2.73], p=0.02).

In the analysis investigating the effect of genotype on the age of first severe exacerbation of asthma, Kaplan-Meier plots sug-

Table 3 Allergic sensitization, eczema and rhinitis by rs7216389 in the cohort

Outcomes	TT	CC+CT	OR (95% CI)	P value
Atopic (SPT) at age 3 (n=673)	38/181 (21.0%)	103/492 (20.9%)	1.00 (0.66-1.53)	1.00
Atopic (SPT) at age 5 (n=723)	53/189 (28.0%)	144/534 (27.0%)	1.06 (0.73-1.53)	0.8
Atopic (SPT) at age 8 (n=717)	57/189 (30.2%)	156/528 (29.5%)	1.03 (0.72-1.48)	0.9
Current eczema at age 3 (n=746)	49/201 (24.4%)	127/561 (22.6%)	1.04 (0.72-1.52)	0.8
Current eczema at age 5 (n=774)	72/201 (35.8%)	166/573 (29.0%)	1.37 (0.97-1.92)	0.07
Current eczema at age 8 (n=762)	49/201 (24.4%)	127/561 (22.6%)	1.10 (0.76-1.61)	0.6
Eczema on physical examination at age 3 (n=744)	30/197 (15.2%)	66/547 (12.1%)	1.31 (0.82-2.09)	0.3
Eczema on physical examination at age 5 (n=764)	37/197 (18.8%)	68/567 (12.0%)	1.70 (1.10-2.63)	0.02
Eczema on physical examination at age 8 (n=734)	31/191 (16.2%)	58/543 (10.7%)	1.62 (1.01-2.60)	0.045
Current rhinitis at age 5 (n=769)	52/201 (25.9%)	163/568 (28.7%)	0.87 (0.60-1.25)	0.5
Current rhinitis at age 8 (n=762)	58/203 (28.6%)	154/559 (27.5%)	1.05 (0.74-1.50)	0.8

TT = mutant homozygote; CC = wild type homozygote; CT = heterozygote

gested an increased risk of severe exacerbation amongst T allele homozygotes (Figure 2), with results of Cox regression analysis indicating an overall hazard ratio of severe asthma exacerbation in this genotype group of 1.53 [1.04-2.27], p=0.03.

Amongst wheezers, there was no significant difference in the risk of having severe asthma exacerbation before or after the first year of life between T allele homozygotes and other genotype groups (p=0.9 and p=0.1, respectively, Table 2).

Allergic sensitisation, rhinitis and eczema

There was no significant association between rs7216389 and allergic sensitisation or current rhinitis at any time point (Table 3). Interestingly, TT homozygotes were more likely to have had eczema present on physical examination at age 5 and 8 years (p=0.02 and p=0.045, respectively, Table 3).

Discussion

Principal findings

This is the first population-based birth cohort study to confirm findings of the association between polymorphisms in rs7216389 SNP and childhood asthma and its severity, and the first study to show the association between this genetic variant and persistent childhood wheezing. We have shown that children with TT genotype of rs7216389 had significantly higher risk of being admitted to hospital for asthma/ wheeze and having severe asthma exacerbations during the first 8 years of life. This risk was specific for hospital admissions occurring after the age of

one year, but not for those in the first year of life. In addition, T allele homozygotes were more likely to have had frequent episodes of wheeze and more likely to be using inhaled asthma medication at age 5 and 8 years. We found no association between T allele of rs7216389 and atopic sensitisation or allergic rhinitis. Although TT genotype was not associated with parentally-reported eczema, it increased the risk of eczema confirmed on physical examination at ages 5 and 8 years.

Limitations and strengths

We attempted to minimize false positive results due to multiple testing, but we acknowledge that the potential impact of multiple testing on the degree to which conclusions can be considered reliable cannot be fully eliminated. Our analysis was hypothesis-driven and limited to a single genotype comparison in several carefully defined phenotypes. Another limitation of our study is that we do not have a functional explanation for our findings.

The most recent GWAS for asthma identified a different SNP (rs2305480) as the SNP most strongly associated with asthma in this region (29). However, we note that rs2305480 is in the same LD block as rs7216389, with a D' of 0.99 (3). We therefore did not genotype rs2305480 SNP for this analysis.

The validity of findings from genetic association studies depends on the quality of phenotypic data used. The major strength of our study is that it is set within a population-based birth cohort with high follow-up rate, which gives higher validity in terms of generalizability of the results. The prospective nature of data collection enabled us to accurately assess the presence of wheeze throughout the first 8 years of life, avoiding the likelihood of recall bias. Another strength is that the availability of data from the children's primary care record gave us access to precise and accurate information on the timing and occurrence of hospital admissions for asthma/wheeze and severe asthma exacerbations.

Interpretation

Our finding that children with TT genotype have an increased risk of wheezing and severe asthma/wheeze exacerbations in early life is consistent with the findings by Bisgaard *et al* (13). In this high-risk birth cohort study, the hazard ratio among T allele homozygotes compared to C allele carriers for asthma was 1.88 [1.15-3.07], severe exacerbations 2.66 [1.58-4.48], and recurrent wheeze 1.64 [1.05-2.59]. However, neither we nor Bisgaard *et al.* (13) found an association between rs7216389 polymorphisms and atopic sensitisation or allergic rhinitis, suggesting that genetic predisposition for asthma differs from that for atopy and allergic rhinitis. We did not find an association between rs7216389 polymorphisms and hospital admissions for wheezing in the first year of life, most likely due to the fact that most children who wheeze in the first year of life have viral infection-induced wheeze and do not continue wheezing beyond the first year (30).

Our finding of the association between rs7216389 polymorphisms and persistent wheezing is comparable with the recent study which found strong association of this SNP with early-onset asthma (0-5 years) (31). In addition, in this study T allele was associated with the severity of early-onset asthma defined on the basis of symptom frequency and medication use. Furthermore, amongst a population of severe asthmatics, the T allele of rs7216389 was associated with childhood asthma-onset disease (32). Similarly, we found that the use of asthma medication was significantly more common amongst children who were T allele homozygotes. Although asthma severity and severe asthma exacerbations reflect different

aspects of asthma control, they both highlight a group of children who may be less responsive to conventional therapy and more susceptible to asthma exacerbations.

What could be a plausible biological mechanism for the observed association? Although there is no clear explanation of the function of the *GSDMB* gene, two studies found a relationship between variants in rs7216389 and *ORMDL3* transcript levels, speculating that rs7216389 SNP could be regulating the expression of the *ORMDL3* gene product (3, 31). The function of the *ORMDL3* gene product has only been recently described (33). *ORMDL3* encodes a transmembrane protein embedded in the wall of the endoplasmic reticulum (ER) which regulates Ca^{2+} influx into ER and is essential for protein folding. Overexpression of *ORMDL3* product inhibits the influx of Ca^{2+} ions into the ER via sarco-endoplasmic reticulum Ca^{2+} pump which results in unfolded protein response (33), and unfolded protein response may cause transcription of genes able to induce inflammation (34).

A recent study of polymorphisms in the 17q21 region and its effect on gene expression levels in cord blood samples of healthy neonates found that T-allele homozygotes of rs7216389 already had an increased expression of the *ORMDL3* gene compared to other genotypes at birth (35). The expression of *ORMDL3* significantly increased after *in vitro* cord blood mononuclear cell stimulation with Der p 1 allergen (35). In addition, T-allele homozygotes for rs7216389 and three other SNPs in 17q21 region had increased baseline IL-17 cytokine production. IL-17 can be secreted by many cell subsets (e.g. T_H17 and T_H2) and has been implicated in pathophysiology of both autoimmune diseases and asthma (36). A proportion of asthmatic individuals who have poorly controlled asthma have increased level of IL-17 in their airways that correlates with the degree of neutrophilic inflammation in their airways as well as airway hyperreactivity (however the exact pathophysiology leading to this type of inflammation remains to be elucidated) (37).

A recent GWA study on white blood cell (WBC) phenotypes found that total WBC and neutrophil counts were strongly associated with chromosome 17q21 region close to *ORMDL3* (38).

We found a significant association between rs7216389 TT genotype and eczema present on physical examination. Apart from one negative report from the Danish high-risk cohort (13), there are no other studies that have looked into the association between childhood eczema and SNPs in *GSDMB* region. This finding warrants replication in other populations.

Conclusion

Our data indicate that children who are T-allele homozygotes for rs7216389 have increased risk of persistent wheezing, are more likely to use asthma medication and to experience severe asthma exacerbations and are more likely to be admitted to hospital because of acute attack of asthma compared to C allele carriers. In addition, amongst asthmatic children, the likelihood of severe exacerbation requiring hospital admission after the first year of life is higher amongst T-allele homozygotes for rs7216389.

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Authors' contributions: Analysis and interpretation of data, drafting of manuscript: ASJ; Conception and design of study, critical revision of manuscript: AC and AS; Genotyping: JH; All authors equally contributed to the final version of manuscript.

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Psychosocial assistance project decreases posttraumatic stress disorder and depression amongst primary and secondary schools students in post-war Bosnia-Herzegovina

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Objective. To assess whether psychosocial support of the School Project of the Humanitarian Society (HS) "Prijateljice (Girlfriends)" had a positive effect on reducing posttraumatic consequences in Bosnia-Herzegovina primary and secondary school students, after the 1992-1995 war. **Subjects and Methods**. A stratified sample of 336 students, aged 13.5±1.6 (10 to18) years, in primary and secondary schools, involved in psychosocial support, were compared with 72 randomly selected peers from the same schools, not involved in this project. Data were collected in December 2005 and in May 2006. The Children's Depression Inventory and the Child Post-Traumatic Stress Reaction Index were utilized. Statistical analysis involved McNemar's test, Students' t-test, Chi-square test and Pearson's correlation test. **Results**. According to DSM, the prevalence of PTSD and depression among students involved in the School Project, significantly decreased from 46.1% to 13.4% and 25.6% to 1.8%, respectively (McNemar's test, P<0.001; P<0.001, respectively). In the control group the prevalence of PTSP and depression decreased from 30.5% to 23.6% and 22.2% to 11.1%, respectively, with no significance (McNemar's test, p=0.332; p=0.077, significantly). Girls had a significantly higher prevalence of both PTSD and depression compared to the boys. Age, the number of traumatic episodes, and suicidal behavior correlated with the intensity of PTSD symptoms and depression symptoms. **Conclusions.** Psychosocial support within the School Project resulted in a significant reduction of PTSP and depression amongst the involved students compared to the controls. Schools and other institutions ought to envisage as many projects as possible to be implemented in school and out-of-school to assist young people to overcome more easily the consequences of the war in their development.

Key words: PTSD, Depression, School Students, Psychosocial assistance, Bosnia-Herzegovina.

Introduction

During and in the aftermath of the 1992-1995 war in Bosnia-Herzegovina (BH), thousands of inhabitants have numerous traumatic experiences (1). By being exposed to serious trauma, many of them developed various psychological disorders, including posttraumatic stress disorder (PTSD) and depression (2-4). Among the most affected groups were soldiers from the front lines, prisoners of war, refugees and displaced persons (1, 4). Together with adults, children were also exposed to serious trauma. In other words, the entire population was exposed to traumatization in a country that was a theatre of war (1, 5-12). Consequences expressed as psychological problems among the population preventing their functioning in everyday activities are present among both adults and youth in school-age. To assist victims of the war catastrophe in BH, together with medical institutions, non-government organizations (NGOs) played an important role by systematically developing and conducting a number of projects for psychosocial assistance to the persons in need. One of them is the Humanitarian Association (HA) "Girlfriends" from Tuzla (13, 14).

The HS "Girlfriends" was registered as a local non-governmental organization in July 1996, by employees, beneficiaries and friends of the international organization "Amica e.V" Freiburg - Germany. The International Organization "Amica e.V." commenced its work in Germany upon receiving the first news about the war in the ex-Yugoslavia. Initial assistance was provided by shipping food, clothing and hygiene goods to Bosnia-Herzegovina and Croatia. In 1994, this organization established a permanent mission in the Tuzla Canton, and the HA "Girlfriends" took over and continued the activities that were already started as a local non-governmental organization in 1996 (13).

Our hypothesis was that psychosocial work with school students who survived war trauma may have positive outcomes in reducing the posttraumatic consequences in this vulnerable population in Bosnia-Herzegovina, in the form of the psychosocial support of the School Project.

Our aim was to estimate whether the psychosocial support of the School Project HA "Girlfriends" had a positive effect on reducing the prevalence of PTSD and depression in Bosnia-Herzegovina primary and secondary school students, after the 1992-1995 war.

Subjects and methods

Subjects

This study involved 408 students aged 13.5±1.7 (10 to 18) years, who volunteered to participate in this research. The sample was divided into the two groups. The first group consisted of 336 students aged 13.5±1.6 (10 to 18) years, who were involved in the "Girlfriends" projects. To avoid ethical conflicts for students in the control group, they were asked and they agreed to participate in this project during the following year (13). This group consisted of 72 randomly selected students aged 13.7±1.9 (11 to 17) years, from the same schools and age-group with no significant age differences (p=0.338), but who were not involved in this NGO's project. Out of 336 students involved in the School Project, 157 (46.7%) were from the Federation of Bosnia-Herzegovina (FBH) and 179 (53.3%) were from the Republika Srpska (RS) entity. There was no statistically significant difference in the number of students in terms of entity origin (p=0.230). During the test period, members of the School Project "Girlfriends" team were present in the classroom.

This „School Project" was approved by both Ministries for Education, Culture and Sport of the Tuzla Canton and the Ministry of Education and Culture of the Republika Srpska. All the procedures and aims of the project were discussed with the head teach-

ers of the elementary and secondary schools involved in the project. Parents gave written informed consent for the children to participate in the study. All participants were informed that participation was voluntary and that they could withdraw from the study at any time.

School project

In 1997, the "Girlfriends" started project implementation in schools located in the Tuzla Canton in order to assist children who had returned from abroad to follow the local curricula. This assistance involved organization of additional lessons for children who had started their education in other European countries in order to overcome the transition period of adjusting to the subjects of their national groups, mathematics, as well as the education system in the Federation of BH in general.

As the two-way (from FBH to RS and vice versa) return process of the BH population to their pre-war places of residence intensified, the project was modified in accordance with beneficiaries' needs. After successful implementation in schools and positive feedback from students, their parents, teachers and school authorities and the positive acceptance of the Ministries for Education, we upgraded the project as late as 2000. The project was given new content in order to support the idea of forming a single school system in Bosnia-Herzegovina. The school project still remains and although the team members changed from time to time, the leader of project is permanently engaged from the beginning up to the present day.

The general aim is to bring about the implementation of a single school system in Bosnia-Herzegovina. Besides the general aim, there are three specific aims:

1. Reduction of prejudice, acceptance of differences, and reduction of aggressive characteristics among children

2. Networking of children, parents, teachers and school management in both entities

3. Formation of Students' Clubs as an official part of the school.

The project: "Supporting returning students in integration into the school system in post war Bosnia and Herzegovina" had the objective of providing education on reconciliation for students, parents, teachers and school managers and support for BH educational reform.

The target groups were students, survivors of war trauma and exile aged 12-15 years, belonging to three different nationalities (Bosniaks, Serbs and Croats) in primary and secondary schools. The parents of involved students, teachers of the involved students, and the school management of the schools were involved in the project.

The project involved 450 students in ten schools, paired according to the partnership of schools in RS and FBH. Every elementary school and secondary school in FBH has a related partner in RS.

The project team included four university educated professionals (one man and three women) in the field of pedagogy, who had regular monthly supervisions with a certified supervisor.

The selection of the primary and secondary schools depended on the concentration of displaced students in certain places in FBH and RS. In FBH we chose five schools (four elementary and one secondary) with the highest number of internally displaced students, who originally belonged to places in RS but they had not returned to their home towns despite the fact that the war had ended. On the other hand we chose five schools in RS (four elementary and one secondary), with the highest number of returnees to their pre-war homes from foreign countries and from FBH.

In a situation where the return process is incomplete, relationships between students of different nationalities in both entities were

difficult, so upon the recommendation of the Organization for Security and Co-operation in Europe - Mission to Bosnia-Herzegovina (OSCE) the "School Project" team applied the project activities to these partners' schools.

The main objectives of this School Project were to give support during the transition period for students moving from one entity to another, so that they could freely continue with their schooling, not losing a school year or having to take additional exams in certain subjects that differ from one entity to another.

Selection of students included in the project

As previously described Hasanović et al. (13) in every school (eight elementary and two secondary) one teacher was engaged with a coordinating role. Teachers formed student groups in their schools using the following selection criteria in both FBH and RS: a) experience of war atrocities and experience of being refugee or internally displaced person, during the war in Bosnia-Herzegovina, b) obstructions and hindrances to return to original homes despite the end of the war, and c) return to reconstructed homes after the war finished in December 1995, with their parents, in and unsafe setting. The exclusion criterion was the lack of war experiences because they left Bosnia-Herzegovina with their families before the war started, and spent the whole war period in a foreign country. Since the project activities were considered to be elective, recruitment was voluntary.

Activities in the Project were adjusted according to the ages of the students:

1. Students' free time activities, over five months, in 20 lessons (every week) were realized in all schools. The issues that were dealt with were: Emotions, Non-violent communication, Peer mediation, Cooperation and tolerance, Stereotypes and prejudices, Children's rights and Humanization of inter-gender relations.

2. Workshops with students (meetings between students from partner schools from both entities, once in one school then in the other), during the observed period twenty workshops were realized (half-day meeting of students) and during those meetings topics from the following fields were covered: Emotions, Non-violent communication and topics about educational reform in BH: "The school I would like".

3. Training for leaders (five students from all ten schools) and for school coordinators, one teacher were chosen from each school. During the observed period two training sessions (15 hours) were held about the issues that contribute to the development of the Student's school clubs: Team work, The role of Student's school clubs in the development of democratic processes in schools, The role of leaders in the Student's school club work and Running initiatives from Student's school clubs.

4. Training for school coordinators (school teachers), during the observed period: three training sessions were held (15 hours) with topics related to "The development of a humane, democratic school".

5. Work with parents:

a) Workshops for parents: in each school one workshop with students' parents was held with the aim of preventing domestic violence: „Parent-child communication"

b) Support for School boards and parents – four workshops were held in two schools (14 hours). The aim of these workshops was to involve parents and students in school management.

6. Work with school management, training sessions were held (five hours) for ten head teachers and ten pedagogues on topics from the field of school and educational reforms in BH.

All activities with students, teachers, school managers and parents were interactive creating a favourable working environment (13). This contributed to the united

school system throughout the territory of Bosnia-Herzegovina. Students initiated eco-activities in their schools, and make efforts to involve other peers. By doing so, they increased their awareness of ecology.

Assessment instruments

We utilized a questionnaire for basic personal and socio-demographical data concerning age, sex, loss of family members, displacement from home and home country, and needs expressed by students. In addition, 20 questions about the students' experiences during the war (15) were included. Even though the students were able to read the questions without significant supervision, we were quite involved and intervened to assist them to reduce the risk of repeated stress and non-identified trauma exposure. It was explained to every pupil that if he or she did not want to answer any of the questions, they were not obliged to. The second questionnaire was the Child Post-Traumatic Stress Reaction Index (16), which measures PTSD symptoms, including forced images, weak concentration and bad dreams. The questionnaire has 16 questions and a score of 7 positive answers is crucial for diagnosing PTSD (15).

The third questionnaire was the Depression Inventory – CDI, which consisted of 27 questions that measured the existing level of depression among children in the 7-17 years age group (17). Every pupil could choose one of three possible answers to any of the questions (e.g. "I do not think about committing suicide", "I do think about committing suicide but would never do it", and "I want to commit suicide"). Responses to all questions are made in a 0-2 scale according to the following principle: 0 – lack of symptom; 1 – weak symptom; and 2 – strong symptom. Informants were asked to evaluate their feelings in the last two weeks, by using the statements offered. The prevalence of depressive symptoms and depression among students was determined on the basis of the CDI cut-off T-score 55.5 (18).

Two testing periods were conducted. The first took place at the beginning of December 2005, whereas the control testing took place at the end of May 2006. Testing on both occasions was conducted by the same examiners, at the same place and under the same conditions.

Statistical analysis

Statistical tests included Student's t-test for differences in age and Spearman's ρ correlation coefficient for prevalence of PTSD and depression. Differences in the frequency of traumatic experiences and socio-demographic data, prevalence of PTSD, depression and suicidal thoughts were tested with the χ^2 test. The level of significance of differences was set to $P<0.05$. Data were statistically analysed using the Statistical Package for Social Sciences, version 10.0 (SPSS Inc. Chicago, IL. USA).

Results

In the group of students involved in the "Girlfriends" School Project there were more girls than boys, 230 (68.5%) out of 336, whereas in the control group there were 37 (51.4%) out of 72 (χ^2-test=7.634, p=0.006). The average age of the participants was 13.6±1.9 and there was no statistically significant difference in the average age of participants between the groups in the sample (t-test=0.713, p=0.476). There was no significant difference in frequency of traumatic experiences among participants, except for destroyed houses, which informants involved in the School Project had significantly more often (p=0.003) (Table 1). However, students involved in the School Project experienced, on average, statistically more traumatic experiences, 7.4 compared

to 5.0 in their peers from the control group (t-test=3.260, p=0.001).

In accordance with the DSM criteria, during the first measuring, PTSD was recorded at total of 43.4% of participants in a total sample. Students involved in the School Project had a prevalence of PTSD 46.1%, which was statistically significant compared to the students (30.5%) who were not involved in this psychosocial project (χ^2-test=5.856, p=0.016) (see Table 2).

In the group involved in the School Project, there was a statistically significant difference in the number of girls, 50.0% out of 230

Table 1 Distribution of adolescents according to frequency of traumatic experiences amongst adolescents in Bosnia and Herzegovina with or without assistance from the Humanitarian Association, ten years after the end of the 1992-95 war

Trauma	Assistance HA (n=336)	Without assistance HA (n=72)	Total (n=408)	χ^2 test	df	p
	n (%)	n (%)	n (%)			
Refugee abroad	62 (18.5)	12 (16.7)	74 (18.1)	0.135	1	0.713
Forced returnee	18 (5.4)	5 (6.9)	23 (5.6)	0.281	1	0.596
Forced to leave home during the war	143 (42.6)	24 (33.3)	167 (40.9)	2.088	1	0.149
Returnee	65 (19.3)	10 (13.9)	75 (18.4)	1.177	1	0.278
Lost family member during the war	150 (44.6)	36 (50)	186 (45.6)	0.686	1	0.408
Mother killed	5 (1.5)	1 (1.4)	6 (1.5)	0.019	1	0.890
Father killed	27 (8.0)	5 (6.9)	32 (7.8)	0.080	1	0.777
Uncle killed	66 (19.6)	12 (15.7)	78 (19.1)	0.340	1	0.560
Grandfather killed	40 (11.9)	5 (6.9)	45 (11.0)	1.487	1	0.223
Close relative killed	43 (12.8)	7 (9.7)	50 (12.3)	0.522	1	0.470
Close person killed	10 (3.0)	5 (6.9)	15 (3.7)	2.637	1	0.104
Good friend killed in the war	39 (11.6)	6 (8.3)	45 (11.0)	0.648	1	0.421
Home destroyed in the war	99 (29.5)	9 (12.5)	108 (26.5)	8.767	1	0.003
Lost someone close to me	149 (44.4)	35 (48.6)	184 (48.1)	0.436	1	0.506

HA= Humanitarian Association "Girlfriends"

Table 2 Prevalence of posttraumatic stress disorder and depression amongst Bosnia-Herzegovina students from both entities, involved and not involved in psychosocial assistance – School Project "Girlfriends"

Diagnosis		n (%) students with diagnosis of PTSD and depression			χ^2-test	df	p
		Yes HA (n=336)	No HA (n=72)	Total (n=408)			
		n (%)	n (%)	n (%)			
PTSD A	yes	155 (46.1)	22 (30.5)	177 (43.4)	5.856	1	0.016
	no	181 (53.9)	50 (69.5)	231 (56.6)			
PTSD B	yes	45 (13.4)	17 (23.6)	62 (15.2)	4.804	1	0.028
	no	291 (86.6)	55 (76.4)	346 (84.8)			
Depression A	yes	86 (25.6)	16 (22.2)	102 (25.0)	0.360	1	0.549
	no	250 (74.4)	56 (77.8)	306 (75.0)			
Depression B	yes	6 (1.8)	8 (11.1)	14 (3.4)	15.561	1	<0.001
	no	330 (98.2)	64 (88.9)	394 (96.6)			

PTSD = Posttraumatic stress disorder; HA= Humanitarian Association "Girlfriends";
A - The first testing took place at the beginning of December 2005; B - The second testing took place at the end of May 2006;
p = the level of significant differences of results between two groups

and boys, 37.7% out of 106 (χ^2-test=4.392, p=0.036). Among the RS students, 49.2% had PTSD, whereas in the Federation of BH, 42.7%, without a statistically significant difference (χ^2-test=1.416, p=0.234).

The second assessment showed 13.4% of students with PTSD in the group involved in the School Project, which is a statistically significant reduction, according to the McNemar test p<0.001. In the group not involved in the School Project, there was also a reduction in the prevalence of PTSP to 23.6%, which is statistically non-significant, according to the McNemar test, p=0.332 (Table 3).

The prevalence of PTSD in the second assessment was statistically more significant among students not involved in the School Project 23.6% compared to those involved: 13.4% (χ^2-test=4.804, p=0.028) (Table 2).

In accordance with the DSM criteria, during the first assessment depression was recorded in a total of 25.0% of informants in the total sample. Students involved in the School Project had a 25.6% prevalence of depression, which was not statistically significant compared to the students (22.2%) who were not involved in this psychosocial project (χ^2-test=0.360. p=0.549) (see Table 2). In the group involved in the School Project, there was a statistically significant difference in the number of girls, 29.1% out of 230, and boys, 17.9% out of 106 (χ^2-test=4.784, p=0.029). Among the RS students, there were 29.6.2% with PTSD, and 21.0% in the Federation of BH, without any statistically significant difference (χ^2-test=3.241, p=0.072).

The second assessment showed 1.8% of students with depression in the group involved in the School Project, which is a statistically significant reduction, according to the McNemar test (Table 3). In the group

Table 3 Distribution of students according to prevalence of diagnosis of depression and PTSD after first and second testing in terms of involvement in the School project of psychosocial assistance in Bosnia and Herzegovina, ten years after the 1992-95 war finished

Involved in the School Project HA "Girlfriends"	Depression second test							
	Depression - first test	Yes	No	Total	χ^2-test	df	p	p*
Yes	Yes	4	82	86	5.411	1	0.020	<0.001
	No	2	248	250				
	Total	6	330	336				
No	Yes	4	12	16	4.018	1	0.045	0.077
	No	4	52	56				
	Total	8	64	72				
	PTSD- second test							
	PTSD- first test	Yes	No	Total				
Yes	Yes	32	123	155	13.047	1	<0.001	<0.001
	No	13	168	181				
	Total	45	291	336				
No	Yes	11	11	22	12.232	1	<0.001	0.332
	No	6	44	50				
	Total	17	55	72				

HA=Humanitarian Association "Girlfriends"; *McNemar test

Table 4 Pearson's correlation "r" of age average, total number of traumatic episodes, severity of depression, severity of PTSD and suicidal thoughts between students (n=408) from primary and secondary schools in both entities in Bosnia and Herzegovina

Severity	Pearson's correlation	Age average	Mean number of traumas	Severity of depression symptoms	Severity of PTSD symptoms
Trauma experiences	r	0.157			
	p	0.001			
Depression symptoms	r	0.116	0.236		
	p	0.019	<0.001		
PTSD symptoms	r	-0.161	0.407	0.342	
	p	0.001	<0.001	<0.001	
Suicidal thoughts	r	-0.024	0.171	0.448	0.192
	p	0.629	0.001	<0.001	<0.001

r=Pearson's correlation coefficient

not involved in the School Project, there was also a reduction in the prevalence of depression to 11.1%, which is statistically non-significant, according to the McNemar test (Table 3). The prevalence of depression in the second assessment was statistically more significant among students not involved in the School Project, 11.1% compared to those that were involved, 1.8% (Table 2).

We found that the age of students was positively associated with the mean number of traumatic experiences and severity of depressive symptoms and negatively associated to the severity of PTSD symptom, while age did not affect fluctuation of suicidal thoughts in students (Table 4). The total number of traumatic experiences was positively associated with depression and PTSD symptom intensity, and suicidal thoughts. Suicidal thoughts were positively associated with the intensity of PTSD and depression symptoms, also the intensity of PTSD symptoms was positively associated with the intensity of depression (Table 4).

Discussion

Our research has shown that at the beginning of the research there were no significant statistical differences among primary and secondary school students in the reports of certain traumatic experiences between the group involved in the School project and the control group, except in cases where the students' houses had been destroyed. The group of students involved in the school project reported statistically more houses destroyed. Regarding survival, the average number of traumas, students from the school group had a significantly higher average than their peers from the control group. All the children had a number of traumatic experiences during the war, especially loss of close family members, which has been described in the literature (1, 4-15, 19, 20)

The presence of PTSD and depression among the students in both entities in North-Eastern Bosnia did not differ statistically. However, the statistics showed that there was more PTSD and depression among the girls than the boys involved in the School project (1, 4), as well as in other research (7-9).

During the second assessment the prevalence of PTSD and depression was reduced statistically in the group where the members of the School project team had worked in compliance with their plan and program of

psycho-social help and there was a reduction in prevailing prejudices regarding acceptance and differences, as well as a reduction in aggressive features among the children, brought about by connecting children, parents, teachers and school management in both entities, as well as the establishment of Students' clubs as part of the schools in which they work.

The intensity of PTSD and depression symptoms were positively associated (4, 21-23) while they also correlated with the number of traumatic experiences and the presence and intensity of suicidal thoughts (4).

Our research has certain limitations taking into account a number of post war traumas that the children were exposed to. Measurement of PTSD and depression levels ten years after the war cannot be an assurance that the obtained results are only based on the consequences of war trauma. The level of PTSP and depression can increase or decrease over a period of time for unknown reasons (1, 4). Little is known about these children and adolescents, the social experiences and additional traumas they had after the war considering the fact that BH is in a transitional period with constant changes of the social framework. This School project justified its existence and may be an example for the development of new similar projects that should be included in all existing primary and secondary schools if possible (13). Further research is needed to understand better the psychological effects of war trauma on young war victims, and the natural course of posttraumatic symptoms, so as to improve interventions targeted at this vulnerable population (14).

Conclusion

This study suggests that work with students on psychosocial support within the School Project resulted in a significant reduction of PTSD and depression prevalence amongst the involved students compared to the controls. Girls presented more severe PTSD and depressive symptoms than the boys. Schools and other institutions ought to envisage as many projects as possible to be implemented in school and out-of-school to assist young people to easier overcome the consequences of the traumatic war in their development.

The findings of this research can be used for recommendations for enhancement and improvement of the primary and secondary educational system, which, besides regular teaching should also have aspects of psychosocial support for traumatized students. Awareness of trauma of students can help teaching staff and school management to develop more efficient approaches in their work with these students instead of a repressive approach.

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Conception and design: MH, SS, MR, MŠ, EH and JH; Acquisition, analysis and interpretation of data: MH, SS, MR, MŠ, EH and JH; Drafting the article: MH; Critical revision for important intellectual content: MH, SS and RH.

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The influence of breast density on the sensitivity and specificity of ultrasound and mammography in breast cancer diagnosis

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Objective. The aim of this study was to analyse the sensitivity and specificity of ultrasound and mammography according to breast density and determine which of these diagnostic imagings is a more accurate test for diagnosis of breast cancer. **Patients and methods.** By means of a cross-sectional study, ultrasound and mammographic examinations of 148 women with breast disease symptoms were analysed. All women underwent surgery and all lesions were examined by histological examination which revealed the presence of 63 breast cancers, and 85 benign lesions. Histological examination was used as the "gold standard". In relation to breast density, the women were separated into two groups, group A: women with "fatty breast" (ACR BI-RADS density categories 1 and 2) and group B: women with "dense breast"(categories 3 and 4). Ultrasound and mammographic findings were classified on the BI-RADS categorical scale of 1-5. For statistical data processing, the logistic regression analysis and the McNemar chi-square test for paired proportions was used. The differences on the level of $p<0.05$ were considered statistically significant. **Results.** In the group of women with breast density categories 1 and 2 the difference in the sensitivities ($p=1$) as well as in the specificities ($p=0.11$) of the two imaging tests was not statistically significant. In the group of women with breast density categories 3 and 4 the ultrasound sensitivity was significantly higher than the mammographic sensitivity ($p=0.03$) without a statistically significant difference in specificity ($p=0.26$). Sensitivity of mammography was (linearly – ex; linearity exists between breast density and the logarithm of odds for a positive result) associated with breast density (likelihood ratio $\chi 2 = 15.99$, $p = 0.0001$). The odds ratio for (the probability of – ex) a positive mammographic result was 0.25 (95% CI, 0.11-0.58). The sensitivity of ultrasound and specificity of each test were not (linearly - ex) associated with breast density. **Conclusion.** Breast density had a significant influence on the sensitivity of mammography but not on specificity. This is very important because a certain percentage of women, not only under 40 but also after 40, have heterogenous and extremely dense breasts (density categories 3 and 4). In these women, ultrasound is a more accurate imaging test than mammography, while in the women with fatty breasts (density categories 1 and 2) these imaging tests are almost equally accurate in breast cancer diagnosis.

Key words: Breast cancer, Breast density, Ultrasound, Mammography.

Introduction

Mammography and breast ultrasound are the most common diagnostic imagings used in breast cancer detection. Mammography is used as a screening method for early detection of breast cancer in women after 40, and as diagnostic mammography in symptomatic women, when a breast lump or nipple discharge is found during self-examination or an abnormality is found during screening mammography (1, 2). Breast ultrasound is used to evaluate specific abnormalities discovered either on mammography or at clinical examination (3, 4).

Although the sensitivity of mammography in the diagnosis of breast cancer is influenced by age, family history, body mass index and some other factors, one of the most important is the inherent limitation of mammography in breast cancer diagnosis is breast density (4, 5). Breast density, which refers to the prevalence of fibroglandular tissue in the breast as it appears on a mammogram, is also associated with increased risk of breast cancer (6, 7). In recent years, the most commonly used method for evaluation and reporting breast density in mammography is the BI-RADS (Breast Imaging Reporting and Data System) proposed by The American College of Radiology (ACR). According to the ACR BI-RADS, breast density is graded on a scale of 1 to 4: (1) almost entirely fat breast; (2) scattered fibroglandular tissue; (3) heterogenous breast, (4) extremely dense breast (8). A global evaluation of breast density and classification leads to the possibility that breast cancer in the mammography image is not revealed. The success of mammography is limited in the group of women with density categories 3 and 4, especially younger women (2, 5). In these two groups there are a number of false negative findings because heterogenous and extremely dense tissue appears white on the mammography image, as does breast cancer ("white breast-white cancer") making it more difficult to distinguish between the two. In contrast, dense glandular tissue usually has a hyperechoic appearance on ultrasound. Because most breast cancers are hypoechogenic, carcinomas in this setting are easily detected on ultrasound ("white breast-dark cancer") (4, 9).

The degree of breast density decreases with age, but a certain percentage of women in menopause have extremely dense breasts. Women who have not given birth or who have had only one child, those who have not breastfed, and women who use contraception or hormone replacement therapy have a higher degree of breast density (10).

The aim of this study was to analyse the sensitivity and specificity of ultrasound and mammography according to breast density and determine which of these two diagnostic imagings is a more accurate test for diagnosis of breast cancer.

Patients and methods

By means of a cross-sectional study, ultrasound and mammographic examinations were analysed of 148 women with breast disease symptoms or positive family history for breast cancer in the period from January 2009 to November 2010. All the women underwent surgery, and all 148 breast lesions were examined by histopathology analysis, which was used as the "gold standard". Histopathology results revealed the presence of 63 breast cancers, and 85 benign lesions. Diagnostic imaginings were performed at the Department of Radiology and Nuclear Medicine, surgical treatment at the Department of Surgery, and pathohistological analysis at the Department of Pathology of the Polyclinic for Laboratory Diagnostics of the University Clinical Centre, Tuzla. The group pattern was made consecutively.

We evaluated the density of breast parenchyma according to the gradation of the

American College of Radiology BI-RADS (10) protocol on a scale of 1-4:

1. almost entirely fat breast
2. scattered fibroglandular tissue
3. heterogenous breast
4. extremely dense breast

In relation to breast density, the women were separated into two groups, group A: women with "fatty breast" (categories 1 and 2) and group B: women with "dense breast" (categories 3 and 4).

The breast ultrasound was performed on a "Sonoline G60 S"- Siemens ultrasound machine with 7.5 MHz-linear array transducer and if needed with a 12 MHz-linear array transducer. Mammography was performed on a Siemens "Mammomat Nova 3000". Standard mediolateral oblique and craniocaudal views of each breast were taken. To obtain and read mammography images, cassettes with phosphorus imaging plates (18x24 and 24x30), mamma-laser drystar DT-2 films and digitizer type CR 85-X with an NX workstation were used. The findings were interpreted by two radiologists. Additionally, according to the radiological features of the described pathological changes, ultrasound and mammographic findings were classified on the BI-RADS categorical scale of 1-5 (4) as follows:

1. no significant abnormality
2. benign finding
3. probably benign finding
4. suspicious lesions- suspicious abnormality
5. highly suggestive of malignancy -malignant lesion

Categories 1, 2 and 3 were considered negative, while categories 4 and 5 were considered as positive for cancer.

Statistical analysis

The standard methods of descriptive statistics (mean and standard deviation) and nonparametric McNemar chi-square test for paired proportions were used for statistical data processing. The sensitivity and specificity of the methods were determined by the 2x2 table diagnostic test. The differences on the level of $p<0.05$ were considered statistically significant. Logistic regression analysis was performed using statistical software MedCalc (version 11.4.2.0). The logarithm of the odds of a positive result was regressed on breast density for cancer patients for each imaging modality to assess the influence of breast density on test sensitivity. The logarithm odds of a negative result were regressed on breast density for subjects without cancer for each imaging modality to assess the influence of breast density on test specificity.

Results

The study included 148 patients, 63 patients with breast cancer and 85 patients with benign lesions determined by the histopathology examination. The mean age of all the patients was 51.6±10.8 years, ranging from 19 to 79 years. The mean age of patients with breast cancer was 55.4±11.4, while the mean age of patients with benign lesions was 48.8±9.4. In the study 36 (24.3%) women were included with almost entirely fat breast (categories 1), 40 (27%) women with scattered fibroglandular tissue (categories 2), 56 (37.8%) women with heterogenous breasts (categories 3), while 16 (10.8%) women had extremely dense breasts (categories 4). Figure 1 shows distribution density categories in different age groups. Dense breasts were found in 87.5% women under 40 years; 70.7% women aged 41-50; 25.5% women aged 51-60 and 18.5% women above 60 years (Figure 1).

In group A (women with fatty breast-density categories 1 and 2) there were 76 (51.4%) patients (mean age 56.1±9.6; ranging from 22 to 79 years). 35 (46.1%) of those women had breast cancer, while 41 (53.9%) women had benign lesions. In this

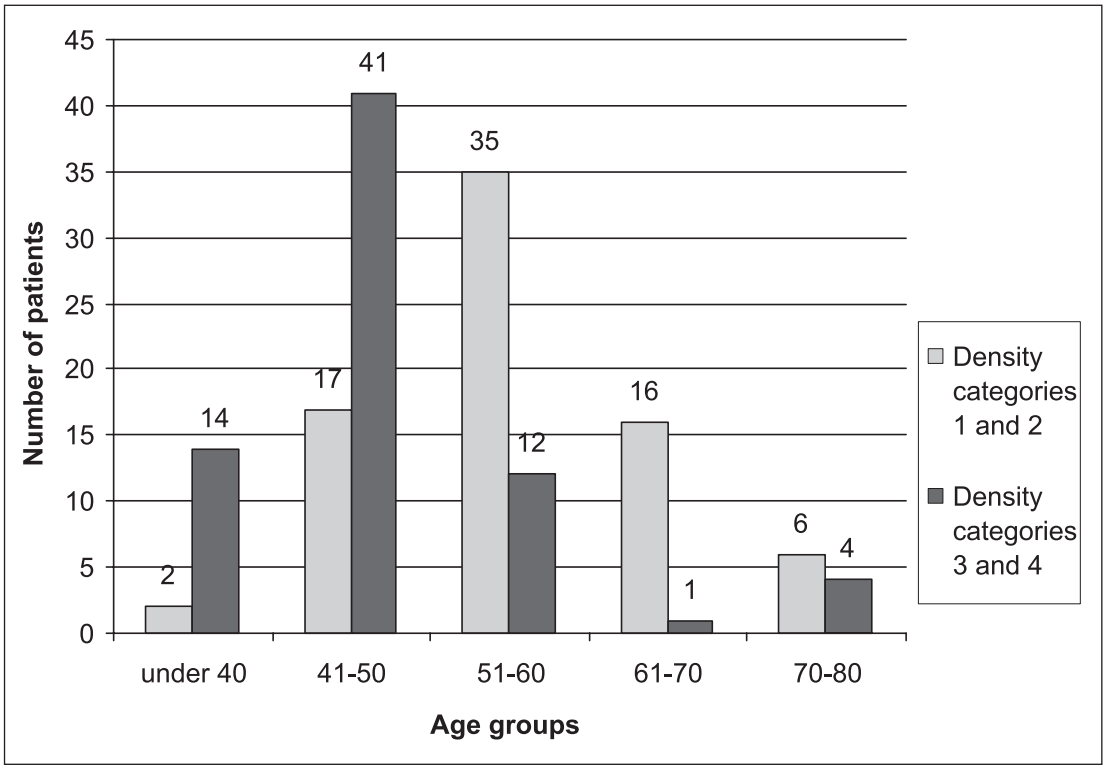

Figure 1 Distribution of breast density categories in different age groups

Table 1 The sensitivity and specificity of ultrasound and mammography in women with fatty breast (density categories 1 and 2)

Reliability	Diagnostic procedures	
	Mammography (95% CI*)	Ultrasound (95% CI*)
Sensitivity (%)	94.3 (80-99)	97.1 (85-99)
Specificity (%)	68.3 (51-81)	82.9 (67-92)
Positive predictive value (%)	71.7 (56-84)	82.9 (67-92)
Negative predictive value (%)	93.3 (77-99)	97.1 (85-99)
Positive likelihood ratio	3 (1.9-4.7)	5.7 (2.9-11.2)
Negative likelihood ratio	0.08 (0.02-0.32)	0.03 (0.004-0.23)

CI*= confidence interval

group, 75% of the women were older than 50 years while 25% women were younger than 50 years. The ultrasound sensitivity in this group was 2.8% higher than mammographic sensitivity, while the specificity of ultrasound was 14.6% higher than mammography (Table 1). In this group the difference in the sensitivities of the two imaging tests was not statistically significant p=1 (χ^2=0). Also, the difference in the specificities of the two imaging tests was not statistically significant p=0.11 (χ^2=2.5) (Table 1).

In group B (women with dense breast-density categories 3 and 4) there were 72

Table 2 The sensitivity and specificity of ultrasound and mammography in women with dense breasts (density categories 3 and 4)

Reliability	Diagnostic procedures	
	Mammography (95% CI*)	Ultrasound (95% CI*)
Sensitivity (%)	53.6 (33-72)	85.7 (67-95)
Specificity (%)	63.6 (47-77)	77.3 (62-88)
Positive predictive value (%)	48.4 (30-66)	70.6 (52-84)
Negative predictive value (%)	68.3 (51-81)	89.5 (75-97)
Positive likelihood ratio	1.5 (0.9-2.5)	3.8 (2.1-6.6)
Negative likelihood ratio	0.73 (0.46-1.15)	0.18 (0.07-0.46)

CI*= confidence interval

(48.6%) patients (mean age 47±10.1; ranging from 19 to 77 years). 28 (38.9%) of these women had breast cancer and 44 (61.1%) women had benign lesions. 76.4% of the women in this group were younger than 50 years, but 23.6% women older than 50 years also had dense breasts.

The ultrasound sensitivity in the women with dense breasts was 32.1% higher than the mammographic sensitivity. In this group the ultrasound sensitivity was significantly higher than the mammographic sensitivity (p=0.03 χ^2=4.92)). The specificity of ultrasound was 13.7% higher than mammography but the difference in the specificities of the two imaging tests was not statistically significant (p=0.26, χ^2=1.25) (Table 2).

The regression of the probability of positive mammographic results on breast density in patients with cancers (sensitivity) was significant (likelihood ratio χ2=15.99, p=0.0001) while the regression of the probability of a positive ultrasound result on breast density in cancers was not significant (likelihood ratio χ^2=1.96, p=0.161). With the increase in breast density, the odds ratio for (the probability of – ex) a positive mammographic result was 0.25 (95% CI, 0.11-0.58), and the odds ratio for (the probability of – ex) a positive ultrasound result was 0.52 (95% CI, 0.19-1.37). These results indicate that a significant linear relationship exists between breast density and the logarithm odds of a positive mammographic result, while there is no linear relationship between breast density and the logarithm odds of a positive ultrasound result.

The regression of the probability of a negative mammographic result on breast density in patients without cancer (specificity) was not significant for mammography (likelihood ratio χ^2=1.23, p=0.27). The regression of the probability of a negative ultrasound result on breast density in patients without cancer was also not significant (likelihood ratio χ^2=0.82, p=0.36). With the increase in breast density, the odds ratio for (the probability of – ex) a negative mammographic result was 0.75 (95% CI, 0.45-1.25), and the odds ratio for (the probability of – ex) a negative ultrasound result was similar, 0.76 (95% CI, 0.42-1.39). These results indicate that no significant linear relationship exists between breast density and the logarithm odds of a negative mammographic or ultrasound result.

Discussion

Women with breast disease symptoms or palpable findings on clinical examination are usually examined by mammography or breast ultrasound or both. The choice of the

primary breast imaging in examining women with symptoms is partly based on age (2). One of the factors leading to false-negative findings on mammography is the effect of breast density (6, 10). Greater breast density is not only related to decreased sensitivity of mammograms because of its masking effect but it is also a major independent risk factor for breast cancer (11). A study of more than 200,000 women showed that the breast cancer risk is 5 times increased in the case of dense breasts compared to women with involutional changes (12). Higher breast density increases cancer risk in addition to the effects of other risk factors, and modifies the effects of body mass index and oral contraceptive use (13). Postmenopausal women with high breast density are at increased risk of breast cancer (14).

The mammographic appearance of the breast tissue varies depending on the tissue composition. Breasts with low density have a high proportion of fatty tissue, whereas breasts with high density have a high proportion of epithelial and connective tissue. In women with heterogenous and dense breasts, dense tissue obscures the radiological picture and makes the identification of cancers more difficult (4, 9, 15, 16).

The advantage of breast ultrasound compared to mammography increases with higher breast density and in young women, where the sensitivity of mammography is low. This is an important fact because more than half of the women younger than 50 years have heterogeneously dense or very dense glandular breast tissue, while one third of women older than 50 years also have dense breasts (17). Kim et al. (18) examined the prevalence of heterogeneous breasts and extremely dense breasts (density categories 3 and 4) in different age groups in Korean women and compared them with known results from western women. In Korean women, the frequency of dense mammogram was 88.1% (30-34 years old), 91.1% (35-39), 78.3% (40-44), 61.1% (45-49), 30.1% (50-54), 21.1% (55-59), and 7.0% (60-64). Korean women in their 40s thus showed a higher frequency of dense mammograms, but this frequency decreased abruptly between the ages of 40 and 54. In western women, there was little difference between 40 and 54 year-olds. It is obvious from these results that a very large percentage of women aged from 40 to 54 belong to density categories 3 and 4 which significantly reduces the sensitivity of mammography, which is used as the screening method after 40. In our study, 87.5% women under 40 years had dense breasts; 70.7% women aged 41-50; 25.5% women aged 51-60 and even 18.5% women above 60 years. These results are similar to Kim's results (18), but in our study, we had more women above 60 with dense breast than Kim et al.

Several studies have shown that detection of breast cancer with mammography is limited in young women and in menopausal women with dense breast tissue (12, 19). Some studies compared the sensitivity of mammography and ultrasound in different density categories (2, 11, 20, 21, 22, 23).

Devolli-Disha et al. (2) in their study conducted on 546 patients with breast symptoms proved that for heterogenous and dense breasts, ultrasound is significantly more sensitive than mammography ($p<0.01$). In their study, mammographic sensitivity in women with predominantly fatty breasts was 82.2% while in the women with dense breasts it was only 23.7%. The specificity of mammography for predominantly fatty breasts was 100%, for heterogeneous breasts 63.5%, while for extremely dense breasts it was only 16.3%. The specificity of ultrasound in their study decreased from 100% in women with predominantly fatty breasts to 72.1% in women with extremely dense breasts.

Other studies, conducted on asimptomatic women, also proved the higher sensitivity of ultrasound than mammography

in women with dense breasts. Leconte et al. (20) found in women with fatty breast 80% of cancers were diagnosed by mammography and 88% by ultrasound. In women with dense breasts the sensitivity of mammography was decreased to 56%, while the sensitivity of ultrasound was still 88%.

In 2002 Kolb et al. (9) published results from a study that was conducted on 11,130 women. In their study, the sensitivity of mammography which was 98% in women with fatty breasts decreased to 48% in women with extremely dense breasts, while the sensitivity of ultrasound in that group was 75%. Crystal et al. (4) in a study of 1517 asimptomatic women, proved the 100% sensitivity of ultrasound in women with dense breasts.

In contrast to previous studies, Barlow et al. (24) reported high mammographic sensitivity even in dense breasts. In their study, the mammographic sensitivity in women with almost entirely fat breasts was 86.3%, in women with scattered fibroglandular densities 90.1%, in women with heterogeneously dense breasts 82.9%, and in women with extremely dense breasts the mammographic sensitivity was 81%. The sensitivity difference of mammography between the different density groups was not high. In their study, the specificity of mammography, which was 92.5% in women with almost entirely fat breasts, decreased to 83.5% in women with extremely dense breasts.

In our study, the sensitivity of mammography was linearly associated with breast density while the sensitivity of ultrasound and the specificity of each test were not. The ultrasound sensitivity in the women with dense breasts was significantly higher than the mammographic sensitivity, while in women with fatty breasts the difference in the sensitivities of the two imaging tests was not statistically significant, which is in accordance with published results (2, 20, 9). The sensitivity of mammography, which was 94.3% in women with fatty breasts, decreased to 53.6% in women with dense breasts, while the sensitivity of ultrasound decreased by 11.4% between these groups. The decrease in the sensitivity of mammography between women with fatty and dense breasts is in accordance with the results of some other studies (2, 9, 20) but not in accordance with the results published by Barlow et al. (24), who found similar sensitivity of mammography in women with fatty breasts and in women with dense breasts. The decrease in the sensitivity of mammography was lower than in the study conducted by Devolli-Disha et al. (2), which was conducted on breast symptomatic women, as in our study. Our results are much more similar to the results of some other studies conducted on asymptomatic women (9, 20). In our study, the difference in the ultrasound specificity between groups was 5.6%, and the difference in the mammography specificity between women with dense breasts and women with fatty breasts was only 4.7%. This last result is only 4.2% lower than the results found by Barlow et al. (24).

Conclusion

Breast density has a significant influence on the sensitivity of mammography but not on specificity. This is very important because a certain percentage of women, not only under 40 but also those in their 40s, 50s and even 60s, have heterogenous and extremely dense breasts (breast density categories 3 and 4). In these women, the ultrasound is a more accurate imaging test than mammography, while in women with fatty breasts (breast density categories 1 and 2) these imaging tests are almost equally accurate in breast cancer diagnosis.

Conflict of interest: The authors declare that they have no conflict of interest. This study was not sponsored by any external organisation.

Authors' contributions: Conception and design: SM; Acquisition, analysis and interpretation of data: SM, MB, HF; Drafting the article: SM; Revising it critically for important intellectual content: SM.

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Psychosocial status of childhood cancer survivors who develop one or more secondary malignancies*

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Objective. Childhood cancer survivors can develop physical, emotional and psychosocial adversities, a secondary malignancy (SM) being one of the most serious among them. The aim of our research was to study whether the development of SM was related to the psychosocial functioning of survivors, especially whether any psychic trauma from the first experience would be aggravated by SM. **Patients and methods.** Seventy – five childhood cancer survivors with SM were matched with 75 survivors who did not develop SM, by sex, age, living enviroment, diagnosis, year of diagnosis and treatment of the first malignancy. They were compared regarding education, employment, marital status and, in the 35 women, childbirth data. Seventeen childhood survivors with an SM had had psychological evaluations at diagnosis of both their first and secondary cancers; the results of the two were compared. **Results.** There were no differences in the schooling, education, social, marital status or birth specifics between survivors with SM and their controls, nor were there marked differences in measures of social or psychological status. **Conclusions.** The socioeconomic status of these 75 subjects was not found to be related to the development of SM. Psychological evaluations showed no marked differences between those conducted after the first and the secondary malignancies.

Key words: Childhood, Secondary malignancy, Psychological evaluation, Socioeconomic status.

Introduction

Approximately two thirds of childhood cancer survivors will display one or more delayed sequelae of treatment (1, 2). Permanent worries about health, problems with schooling, employment and complicated relations in the family may negatively influence their quality of life (QOL) (3). There are, however, some investigators who report that childhood cancer survivors have the same QOL as their peers: they find as many married, have the same number of children and undergo no more divorces when

compared to the general population (4, 5). It is not clear either, whether depression and suicide rates among childhood cancer survivors are higher (6, 7). There are, moreover, inconsistencies in QOL reports suggesting to some that survivors may be biased in their responses to questionnaires (8-10). There is also a lack of comparability across studies due to wide variations in study designs. More information regarding these issues is needed (11). We therefore report the psychosocial and psychological functioning of childhood cancer survivors who developed SM as one of the important determinants influencing their quality of life.

Somatic late sequelae as well as cognitive and psychosocial functioning have been extensively analysed and reported (12, 13). Cognitive, emotional, behavioral and psychosocial problems are the most common. Disturbances of concentration, attention span and memory could be the result of any one of many causes and their interactions with individual idiosyncracies including age at diagnosis and treatment administered (14, 15). Among the somatic adversities, SM is one of the most serious. Development of SM in the survivor might also awaken painful memories of the first malignant tumor and all it entailed. We did not find any reports comparing psychosocial functioning of childhood cancer survivors who developed SM with those who did not.

The aim of this study was to find out whether the development of SM was related to the psychosocial functioning of survivors, and if any negative sequelae after the first diagnosis became even stronger and deeper after the diagnosis of SM. We compared the socioeconomic status of 75 childhood cancer survivors who had SM and of 75 matched survivors who did not. We further compared the findings of psychological testing of 17 childhood cancer survivors who were assessed both after the first cancer was found and after SM. Moreover, their findings were compared with 17 survivors who did not develop SM.

Patients and methods

Patients

In Slovenia, obligatory registration of all cancer patients became established in 1950 with the advent of the Cancer Registry of Slovenia. Treatment of children with cancer is centralized at the Children's Hospital. After treatment, all are followed up by the same center for at least five years or until they are 18 years old. Later they are followed up regularly at the outpatient Clinic for Late Effects at the Institute of Oncology, Ljubljana. This policy has been in effect since 1986 (2). The patients enrolled in our studies are seen at least once every year for evaluation of their somatic and social status. In most of them their psychoemotional status is also evaluated.

Between 1959 and 2006, 1984 children with cancer were registered at the Cancer Registry in Slovenia. Of the 102 who developed one or more SM, 27 died. The remaining 75 patients with SM, 35 women and 40 men, have been followed up regularly. For the present study, they were matched with 75 cancer survivors who did not develop SM (control group). Matching was done by stratifying the register population by year at diagnosis of the first malignancy, and by sex, age, and diagnosis and treatment of the first cancer. From each stratum, individuals were selected randomly. The age at diagnosis of the first malignancy of both groups ranged from 0 to 16 years, with a mean age of 8.4 (standard deviation, SD=5.1) years for survivors with SM as well as for controls. The mean age at last evaluation (December 2009) was 34.9 (SD=8.7) years for survivors with SM and 33.9 (SD=7.7) years for controls. The diagnosis of the 1st malignancy in survivors with SM and their controls was leukemia in 25.3%, Hodgkin disease (HD;

Table 1 Development of second malignancy in 75 survivors

Diagnosis of first malignancy, n (%)		Diagnosis of second malignancy, n
Leukemia	19 (25.3)	Other Carcinomas 4, Brain 3, NHL 3, HD 2, Malignant bone tumors 2, Testis 2, Soft-Tissue Sarcomas 1, Thyroid Carcinoma 1, Leukemia 1,
Brain	11 (14.7)	Brain 7, Thyroid Carcinoma 2, Other Carcinomas 2
HD	15 (20.0)	Thyroid Carcinoma 9, Other Carcinomas 5, Malignant melanoma 1
NHL	5 (6.7)	Other Carcinomas 3, Brain 1, Thyroid Carcinoma 1
Renal tumors	3 (4.0)	Thyroid Carcinoma 2, Brain 1
Neuroblastoma	4 (5.3)	Thyroid Carcinoma 2, Rhabdomyosarcoma 1, Other Carcinomas 1
Soft-Tissue Sarcomas / Rhabdomyosarcoma	5 (6.7)	Other Carcinomas 4, Soft-Tissue Sarcomas 1
Malignant bone tumors / Ewing's & PNET	5 (6.7)	Malignant bone tumors 2, Other Carcinomas 2, Leukemia 1
Gonads	3 (4.0)	Soft-Tissue Sarcomas 1, Ovary 1, Other Carcinomas 1
Thyroid Carcinoma	1 (1.3)	Soft-Tissue Sarcomas 1
Other Carcinomas	2 (2.7)	Thyroid Carcinoma 1, Other Carcinomas 1
Retinoblastoma	1 (1.3)	Soft-Tissue Sarcomas 1
Unspecified malignant neoplasms	1 (1.3)	Thyroid Carcinoma 1

HD = Hodgkin's disease; NHL = non-Hodgkin's lymphoma; PNET = primitive neuroectodermal tumor

20%), brain tumors (14.7%), non-Hodgkin lymphoma (NHL; 6.7%) and remaining conditions (33.3%). The great majority of patients were treated with both irradiation and chemotherapy (49.3%), others had surgery and irradiation (15.3%) or surgery and chemotherapy (10.7%), 13.3% of patients were treated with all three methods, and a few had only one (11.3%). All 150 survivors were assessed five or more years after treatment. The SM in the 75 survivors are presented in detail in Table 1. The most common SM were other carcinomas (23; 30.7%), thyroid carcinomas (19; 25.3%) and brain tumors (12; 16.0%), which developed in irradiated areas. Five soft tissue tumors (6.7%) and miscellaneous other types accounted for the rest. The third tumors were either carcinomas (8) or brain tumors (4), all in irradiated areas.

The study was performed after approval by our Human Investigations Committee and in accordance with the precepts of the Helsinki Declaration. Informed consent was obtained from each participant in the study and it was concluded in December 2009.

Measurements

Evaluation of socioeconomic status

Survivors included in this study had previously been evaluated and analyzed as to their late somatic sequelae including: neurological deficits (16), endocrine deficits (17), renal function (18), cardiac status (19), SM (20), and psychosocial status (21). For the present study, the group of survivors with SM and the control group were compared as to their living environment, education, employment, marital status, and, in the 35 women, childbearing. Living environment was listed as city, town or countryside, following official Slovene definitions according to the number of inhabitants (>100000, 3000-100000, and <3000 inhabitants, respectively). The influence of rural versus urban environment might become impor-

tant for the survivors in their future life. In the city, opportunities for schooling and education are better than in our countryside where, in contrast, marital status may assume more importance.

Psychological evaluation

The psychological status was evaluated in the second part of our study. It was performed on a voluntary basis; not all patients responded to our invitation. Twenty-nine of the 75 with SM had had psychological evaluation after the diagnosis of the first malignancy; they were invited to participate in a follow-up psychological assessment and 17 responded. In them, the first psychological evaluation had been performed 9 to 32 years before (mean=15.9, SD=5.2). The survivors without SM were tested 7 to 28 years (mean=15.2, SD=5.3) after the diagnosis of the first cancer. The mean elapsed time between the first and the second psychological tests for those with SM was 12.7 (SD=4.5) years, with a minimum of 5 years and maximum of 22 years.

Seven men and 10 women with SM, and their controls were evaluated. To be able to compare results, the same tests were employed as those used several years before. These were the standard Bender visual motor gestalt test (22, 23) for evaluating visual-motor functioning and visual perception skills, the Rorschach test (24) for personality characteristics and emotional functioning, and the Wechsler Bellevue test (25) for intelligence. The presence and the degree of any psychoorganic syndrome or emotional disorder, if present, was determined on the basis of the qualitative evaluation of the results of all three instruments. The term *psychoorganic syndrome* in this context includes disturbance of concentration, memory, learning processing or intellectual efficiency. *Emotional disorder* here includes emotional instability, dullness and a lower ability to adapt to changing circumstances. Both psychological characteristics were classified by the same expert into four categories: 0 - no disorder; 1 – disorder present; 2 – significant disorder; and 3 – very significant disorder. From our results we determined whether a disturbance was present in an individual and, if so, its severity. These semi-objective results, however, had to be evaluated subjectively as well since they are not amenable to mathematical analyses.

Following the hypothesis that the diagnosis of SM could affect emotional functioning, either in increasing anxiety, or deepening depression, the Plutchik Profile Index Emotions (26) was used at our second examination in the 17 patients with SM. Their results were compared with the norms valid for the general population of Slovenia (27).

Statistical analysis

Numerical data are presented as mean and SD and categorical data as proportions. To assess the difference between the observed and expected frequencies for categorical variables, the Pearson's chi-squared test, Fisher's exact test or Freeman-Halton extension of the Fisher's exact test was applied, as appropriate. In order to compare the psychological status and the type of tumor, the psychoorganic syndrome and emotional disorder variables were defined as ''no disorder" and ''disorder" present, while the diagnosis of the first malignancy was categorized as ''brain tumor" and ''other tumor". A p value of <0.05 was considered significant. Data were analyzed using the PASW 18 software (SPSS Inc., Chicago, IL, USA).

Results

Evaluation of socioeconomic status

There was no difference in the environment where the patients with SM or the controls were living ($\chi^2_{(2)}=0.33$, p=0.863) nor in the

Table 2 Social status in SM and control group

Variable	SM			Control			P*
	Male n=40	Female n=35	All n=75	Male n=40	Female n=35	All n=75	
Residence, n (%)							0.863
City	7	2	9 (12.0)	7	0	7 (9.3)	
Town	10	13	23 (30.7)	13	12	25 (33.3)	
Country	23	20	43 (57.3)	20	23	43 (57.3)	
Marital status, n (%)							0.839
Married / living with partner	9	12	21 (28.0)	7	12	19 (25.3)	
Single / living with parents	30	20	50 (66.7)	33	20	53 (70.7)	
Living alone	1	3	4 (5.3)	0	3	3 (4.0)	
Education, n (%) (12 have not completed schooling)							0.300
Grammar	7	8	15 (20.0)	5	2	7 (9.3)	
Vocational	10	7	17 (22.7)	12	7	19 (25.3)	
High	12	10	22 (29.3)	11	9	20 (26.7)	
College	3	3	6 (8.0)	2	3	5 (6.7)	
University	8	6	14 (18.7)	8	12	20 (26.7)	
Auxiliary	0	1	1 (1.3)	2	2	4 (5.3)	
Employed, n (%) (138 with completed schooling)							0.818
In their profession	21	20	41 (59.4)	19	22	41 (59.4)	
Other	6	3	9 (13.0)	9	3	12 (17.4)	
Unemployed	5	5	10 (14.5)	3	4	7 (10.1)	
Retired / social support	3	6	9 (13.0)	4	5	9 (13.0)	
Children, n (%)†							0.947
0	32	20	52 (69.3)	33	19	52 (69.3)	
1	5	6	11 (14.7)	3	9	12 (16.0)	
2	2	8	10 (13.3)	3	5	8 (10.7)	
3	1	0	1 (1.3)	0	2	2 (2.7)	

SM= secondary malignancy; * Comparison of social status variables with regard to patient group.
† One answer is missing from a female patient (SM group) and another from a male patient (control group).

marital status of the two groups (p=0.839) (Table 2).

Most patients in both groups were still single and living with parents (67% and 71%). About one quarter of both groups were married or living with a partner. Although there were some differences between the SM and control group for particular levels of education, these did not attain statistical significance (p=0.300). The employment status of the 2 groups was comparable (p=0.818). Most (around 60%) were working. In each group there were eight who had retired (for disability) because of severe adverse sequelae after treatment for either the first or subsequent cancer(s). Five of the 8 retired survivors with SM had had a primary brain tumor. Another had a bone tumor

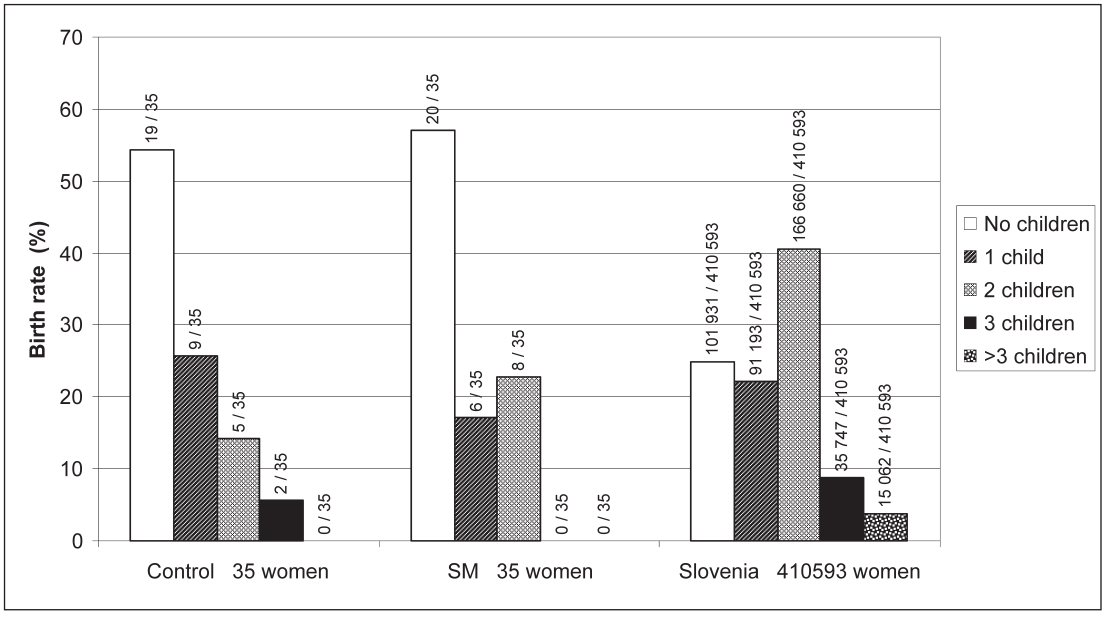
with amputation and later a disarticulation for a secondary tumor and a pneumonectomy for metastatic disease. Two had HD with severe sequelae after radiation therapy (RT) to the mediastinum (one also had a secondary breast cancer).

Three of the 8 retired subjects in the control group had a primary brain tumor; one with Ewing's sarcoma had severe sequelae after chemotherapy (ChT) and 50 Gy RT to the pelvis. Another had hypertension and epilepsy after surgery and ChT for a malignant tumor of the suprarenal gland, and three others had pulmonary fibrosis and heart failure after RT to the mediastinum for HD.

One survivor in each group receives the social support, being disabled after treatment of a primary brain tumor. Comparison of the study groups according to the number of children born, showed very similar proportions (p=0.947). Most (69%) were childless: 32 men with SM and 33 in the control group, as were 21 women with SM and 19 in the control group.

The proportion of women who gave birth of the general population of women in Slovenia in the year 2002 was 75.2% (27). The childhood cancer survivors in both groups gave birth in significantly lower proportions than the general Slovene population: 40.0% for the SMs, and 45.7% for the controls (p<0.0001). Furthermore, 40.6% of Slovene women had 2 children in 2002. This is a significantly higher proportion than that of the SM women (22.9%, p=0.033) or those of the control group (14.3%, p=0.002) (Figure 1).

Twenty-two of 150 childhood cancer survivors (14.7%) had brain tumors and 128 (85.3%) had other diagnoses. Comparison of socioeconomic status and the presence of a brain tumor (brain/other tumors) showed statistically significant relationships with education (p<0.001) and employment (χ^2=15.52, p<0.001). About 2 of 3 non-brain-tumor patients finished high school, college or university studies (83/128, 64.8%), while perhaps only one in five of those with a brain tumor attained comparable educational levels (4/22, 18.2%). With regard to employment, only 8/21 (38.1%) patients with a brain tumor were employed, contrasted with 76/117 (65.0%) of those with



SM=Secondary malignancy.

Figure 1 Women 22-49 years old, birth rate

some other malignancy. The unemployed or retirement rate was 13/21 (61.9%) for brain tumor subjects, and 18/117 (15.4%) for those with other primary diagnoses.

Psychological evaluation

The results of psychological evaluation of the 17 childhood cancer survivors with SM and 17 controls are shown in Table 3.

There was no significant difference in the frequency or severity of the psychoorganic syndrome or emotional disorder rankings between the SM group at the second evaluation and the control group (p=0.382 and p=0.265, respectively). Table 3 shows that there was no significant difference, within the cognitive and emotional spheres, between the two groups. This table also shows that there are no differences within the group of individuals with SM at the first as compared to the second examination. Disturbances of concentration and attention, shortening of attention span and weakness of memory were already present at the first evaluation, and visual-motoric incoordination was also observed. Notably, however, intellectual functioning (with the exception of one woman) was at least in the mean range, possibly even above it.

The second psychological evaluation of psychoorganic syndromes and the emotional sphere did not show any significant differences as compared to the results of the first (p=1 and p=0.999, respectively). Early changes were observed in 13 subjects; they included some personal characteristics, interpreted as aggravated anxiety and a diminished capacity for normal aggressiveness and assertiveness. Comparison of psychological status (no disorder/disorder) and the presence of a brain tumor (brain/other tumors) showed significantly more instances of the psychoorganic syndrome in patients with brain tumors as the first malignancy than in those with other tumors (4/4 versus 10/30 (p=0.022), respectively). However, no such clear relationship was found when the SM was a brain tumor ; i.e., 3/5 patients with a brain tumor and psychoorganic syndrome versus 3/12 with some other secondary tumor (p=0.280). For emotional disorders, there was no evidence of any relationship with the type of tumor: 3/4 versus 24/30 patients for the first malignancy (p=1). The proportion of psychoorganic syndrome was

Table 3 Psychological evaluation at diagnosis of first and of second malignancy

Psychological disorder	SM (n=17)		Control (n=17)	P 1st Dg / 2nd Dg	P 2nd Dg / control
	At 1st Dg	At 2nd Dg			
Psychoorganic syndrome, n (%)				1	0.382
0	11 (64.7)	11 (64.7)	9 (52.9)		
1	2 (11.8)	2 (11.8)	3 (17.6)		
2	3 (17.6)	3 (17.6)	1 (5.9)		
3	1 (5.9)	1 (5.9)	4 (23.5)		
Emotional disorder, n (%)				0.999	0.265
0	2 (11.8)	1 (5.9)	5 (29.4)		
1	11 (64.7)	11 (64.7)	8 (47.1)		
2	4 (23.5)	5 (29.4)	4 (23.5)		
3	0	0	0		

Dg = diagnosis; SM = secondary malignancy; 0 = no disorder; 1 = disorder present; 2 = significant disorder; 3 = very significant disorder

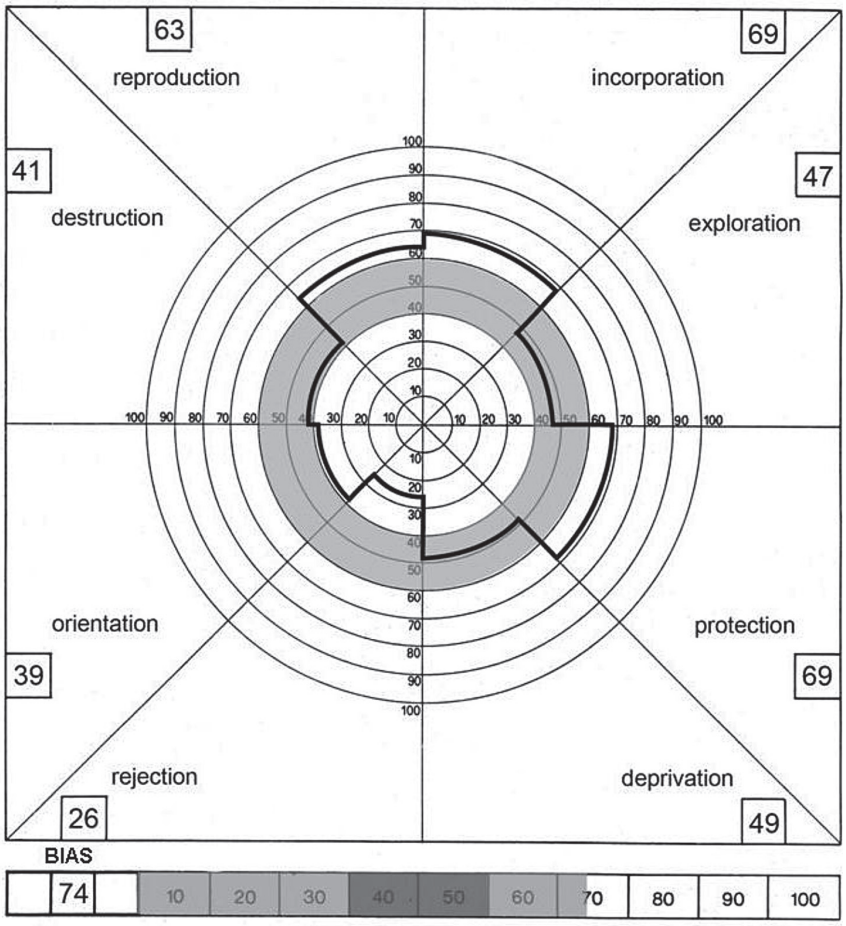


Figure 2 Plutchik Profile Index Emotions in survivors with SM. Scores for 8 emotional dimensions are presented in percentiles. Grey areas represent normal values (range 40-60). Average scores for emotional dimensions are shown in frames in each of 8 fields and illustrated with a bold black line.

4/5 versus 12/12 (p=0.294) for the SM patients with brain tumors versus those with other tumors, respectively.

The result of the Plutchik Profile Index Emotions instrument was used in SM patients at their second psychological examination, in an attempt to find out how these individuals function on average. The results are shown in Figure 2.

Units 0 to 100 are percentile. Average scores of particular emotional dimensions are transformed, with the help of testing tables, into percentile scores. Values 40 to 60 are normal. The grey areas represent normal values, the uninterrupted black line shows the mean value of the 17 evaluated patients with SM, and the individual values are very close to each other. This profile suggests that these 17 survivors with SM see themselves as socially adjusted with only a few of them markedly inverted, isolated or distrustful (*Reproduction* and *Incorporation)* perhaps along with some diminished elasticity and adaptability to new circumstances, as indicated by the next characteristic *Protection*. This is elevated, suggesting a fear of not being able to deal with future difficulties. The low average for the dimension *Rejection* also

corroborates this point of view, indicating that those tested were probably less decisive and less self-sufficient than the general population. They might lack fighting spirit and enterpreneurship. This, in turn, is corroborated by the next two personality dimensions: *Orientation* and *Destruction*. The first one suggests a marginally lower capacity of planning for the future due to a perceived lack of self-control. The second dimension emanates from the first one, namely, a certain lack of healthy aggressiveness and life-enhancement. Scores for the 17 were at the lower average levels. Notably, such individuals do not feel particularly sad or depressed, and have, on average, no more sense of being marginalized, deprived or lonely than the general population (*Deprivation*). They try to adjust to new circumstances just as those without their experience *(Exploration*). The elevated values on the dimension *Bias* suggest that they are trying to show themselves in the best possible light.

Discussion

Impaired quality of life for childhood cancer survivors has been reported (28, 29). We hypothesized that the advent of SM in them would exacerbate or worsen pre-existing psychosocial difficulties, but this did not prove to be the case.

Our research encompassed several QOL indices. One of these was employment status. The same proportion of SM subjects and their controls were working or had undergone early retirement because of severe disabilities related to treatment of the first or SM. These characteristics resulted in diminished competitiveness and impaired capacity for personal contacts. They were pronounced in four of the 13, and prevented ordinary socializing with their peers; these four were still living with their parents.

The birth rate is another QOL measure, and we found no detectable difference in childbearing among those with SM and their controls. Somatic dysfunctions did not fully explain the fact that few children were born in either study population. Other reasons, such as socioeconomic and emotional, must be invoked to explain the findings (29). It is of interest, however, that childhood cancer survivors with SM had no more than two children, whereas in the control group there were some women with three (Figure 1). For all 305 female childhood cancer survivors in Slovenia (out of 1984 registered children with cancer between years 1959 and 2006), the proportion that was childless was significantly higher than in the general population (68.5 vs. 24.8%, $p<0.0001$). There may be somatic reasons why childhood cancer survivors remain childless. For example, we found hypogonadism in 35, and sterility in 9 of our long term survivors. Even when those 44 women were excluded, the birth rate was still low (165/261, 63.2%).

We did not find any marked differences in psychological deviations between the 17 survivors with SM and their controls; neither did the second psychological evaluation of the emotional sphere show any marked differences as compared to the results of the first evaluation. The results of the Plutchik Profile Index Emotions, used only in those with SM, showed that they feel, on an emotional footing, equal to the general population. This suggests that those who cope with the primary malignancy can successfully avoid later psychoorganic or emotional disturbances. It is noteworthy that they do not tend to be sad or depressed or have any sense of emotional, social, or material deprivation. This may be indicative of a strong innate defense against any anxiety that might be caused by either the primary cancer or SM. That inner strength could lead to unwarranted optimism that, however, should not be discouraged in our opinion. It is considered by us to be of great help in lives marred by uncertainties.

The group of patients with SM, who were psychologically evaluated twice, is small, numbering but 17, which might be a significant limitation to this study. Such study populations are rare, however, and worthy of record. The uniformity of the results among them tends to be convincing as is the comparison of the 75 SM survivors with their control group. It might be expected that this report will encourage studies on larger patient populations.

Adding to the credibility and interest of the data reported here is the unusually long follow–up time plus the fact that the same psychologist and oncologist evaluated the psychological and socioeconomic functioning of the patients (30). This continuity is favored by our system of smoothly shifting patients from the pediatric to the adult follow up clinic (31, 32).

A limitation of our study is the absence of an independent validation of the classification of psychoorganic syndromes and emotional disorders.

Circumstances contributing to our results might be our system of rehabilitation in groups, promoting social interaction and mutual help. We have established the Foundation »The Little Knights« supporting survivors of childhood cancer (33). This takes care of some of their needs, both material and psychological. As a result of our positive experience with this work it might be recommended as a model to help childhood cancer survivors.

Conclusions

There seems to be no difference in the educational level or the marital or employment status between survivors of childhood cancer who do or do not develop SM. When compared with the number of children born to the general population of women in Slovenia, childhood cancer female survivors in both groups have significantly fewer children. The second psychological evaluation in the emotional sphere of the childhood cancer survivors with SM showed no marked differences from the results of their first tests performed years before. Subjects with SM do not feel particularly sad or depressed, and have, on the average, no more sense of being marginalized, deprived or lonely than the general population. They try to adjust to new circumstances just as those without their experience, and try to show themselves in the best possible light.

Addendum

An illustrative case

Statistical data are important, but personal observations are of value, too. A young women had this to say about herself at one of our group sessions: "I was always very shy as a child, but my self-confidence grew when I first fell ill. I found I could confront my first cancer --- Hodgkin's disease --- and all that went with that diagnosis. I could discuss matters with the doctors, family, friends and other patients in the clinic. When I was 28 and developed signs of my secondary cancer, I had to do things by myself. My self-confidence then proved to be important because my family physician did not take my concerns seriously. He sent me to a psychiatrist. I decided not to listen to him and went instead to the Late Effects Clinic at the Oncology Institute. They made the diagnosis and treated me." Her secondary malignancy was breast cancer, and she has now been disease-free for 12 years.

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Conflict of interest
The authors declare that they have no conflict of interest.

Authors' contributions:
Conception and design: BJ; Acquisition, analysis and interpretation of data: RK and MČS; Drafting the article: RK and MČS; Critical revision for important intellectual content: GD'A.

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Markedly elevated plasma D-dimer and the prevalence of acute pulmonary embolus

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Objective. To evaluate if increasingly elevated levels of plasma D-dimer are associated with higher prevalence of acute pulmonary embolus (PE). **Patients and Methods**. A retrospective study was conducted evaluating all PE protocol CT examinations performed in low-to-intermediate risk emergency department and hospitalized patients during 2007. All PE protocol CT reports were reviewed for the presence or absence of acute PE. The charts of all of these subjects were then reviewed for quantitative plasma D-dimer values, measured in mg/ml Fibrinogen Equivalent Units, drawn within one day prior to the CT exam. The prevalence of acute PE at different D-dimer threshold results was then evaluated using D-dimer groups as follows: < 1.0 mg/ml, ≥ 1.0 but < 2.0 mg/ml, ≥ 2.0 but < 4.0 mg/ml, and ≥ 4.0 mg/ml. **Results**. 943 PE protocol CT exams were reviewed. 410 subjects had D-dimer values drawn before their CT exams; 30 (7.3%) of these were positive for acute PE. As D-dimer values became increasingly elevated, the prevalence of acute PE increased accordingly. In particular, D-dimer elevation ≥4.0 mg/ml was almost 94% specific for acute PE by CT criteria. **Conclusion**. Increased elevation of plasma D-dimer is associated with increased prevalence of acute PE in low-to-intermediate risk patients.

Key words: Pulmonary embolus, D-dimer, CT.

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Introduction

Acute pulmonary embolism (PE) remains a critical diagnosis, and the need for rapid diagnosis of acute PE is similarly critical. The current standard diagnostic method for diagnosing acute PE is through the use of multidetector contrast-enhanced CT using thin-slice reconstructions (PE protocol CT) (1). Various clinical algorithms for the evaluation for acute PE exist (2, 3). These algorithms, in conjunction with plasma D-dimer values, are frequently used to help classify patients as high or low risk for acute PE. Prior studies demonstrate that "normal" values of D-

dimer within the reference interval predict successfully a very low probability of thromboembolic disease and effectively exclude acute PE in low-to-intermediate risk patients. However, as it is known to be elevated in a variety of conditions, borderline elevated D-dimer levels are not very specific for the presence of PE (4). To our knowledge, there have been no recent studies evaluating the specificity of markedly elevated plasma D-dimer levels for the presence of acute PE.

The aim of this study is to evaluate the prevalence of acute PE by CT criteria in low-to-intermediate risk hospitalized and emergency department (ED) patients with markedly increased plasma D-dimer values; specifically, if acute PE prevalence is higher in such patients with greater D-dimer values.

Patients and methods

Patients and data collection

After approval from our Institutional Review Board, and in compliance with the Health Insurance Portability and Accountability Act, a retrospective review of all reports from CT examinations performed at a single urban teaching hospital between January 1, 2007 and December 31, 2007 was conducted to search for PE protocol CT exams. The PE protocol CT exams were found by searching the radiology CT report database using the keywords "PE", "pulmonary embolus", and "pulmonary emboli". Of the retrieved reports, only those for PE protocol CT exams were considered; reports from aortic dissection protocol CT or standard contrast enhanced chest CT examinations that incidentally discovered PEs were excluded. Studies performed in patients under the age of 18 and those performed on a strict outpatient basis were also excluded. The collected reports were then reviewed for the presence or absence of acute PE. A report was considered positive for acute PE if (1) at least one pulmonary embolus was present, and (2) there was no description in the report to suggest that the embolus had been present on a prior examination or was chronic in appearance. Any limitations to the diagnostic quality of the CT exams noted in the radiology reports (i.e., patient respiratory motion, poor opacification of the pulmonary arteries, etc) were also tabulated.

Imaging

For the data included in this study, PE protocol CT exams were performed on 16 channel and 4 channel multidetector CT scanners (Siemens Medical Solutions, Erlangen, Germany). All images were reconstructed to 2 mm slices for interpretation on a PACS workstation.

A volume of 75-150 ml low-osmolar iodinated contrast material (iohexol, Omnipaque 300, GE Healthcare, Waukesha, WI, USA) or iso-osmolar iodinated contrast material (iodixanol, Visipaque, GE Healthcare) was administered at 4 ml/s via a power injector (MEDRAD, Inc, Warendale, PA, USA). A region of interest was placed over the main pulmonary artery, and image acquisition was triggered once the average density within the region of interest exceeded a threshold of 90 Hounsfield Units (bolus tracking). All exams were interpreted by board certified radiologists, and the documented PE protocol CT result as described above was based on this reading.

D-dimer data collection

Once all the PE protocol CT reports were obtained, the charts of all subjects were reviewed for all available D-dimer testing. Any D-dimer values obtained on the day of or the day prior to the PE protocol CT examination were documented. Our institution utilizes a commercially available, automated, immuno-turbidimetric assay with latex microparticles (Diagnostica Stago, Paris, France) which provides fully quantitative

D-dimer values between the ranges of 0.22 and 20.0 μg/ml. The upper limit of normal for D-dimer values (in Fibrinogen Equivalent Units or FEU) at our institution in 2007 was 0.4 μg/ml. Internal validation of this D-dimer assay performed in 2003 at our institution in normal adults (n = 62) yielded (in μg/ml): mean = 0.30; 1 standard deviation = 0.10; 2 standard deviations = 0.21; median = 0.29; range = 0.15 – 0.50.

Statistical analysis

Once all data were tabulated, the sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of the ability of D-dimer to predict the presence of acute PE on CT were calculated at D-dimer values of <1.0 μg/ml, ≥1.0 but <2.0 μg/ml, ≥ 2.0 but <4.0 μg/ml, and ≥4.0 μg/ml. Appropriate 95% confidence intervals were also generated. Pearson's χ^2 analysis was employed to test the null hypothesis that prevalence of acute PE was independent of D-dimer value. A p-value of <0.05 was considered statistically significant.

Results

Acute PE prevalence

962 PE protocol examinations were performed in our hospital in 2007. One examination was excluded from this study as the patient was under 18 years of age, and 18 additional examinations performed on non-emergency outpatients were also excluded. Therefore, 943 CT examinations, from a total number of 883 subjects (343 male; ages ranging from 18 to 99 years), were included in the final analysis. All subjects were either inpatients or ED patients. 87 examinations (9%) were positive for acute pulmonary embolus. Of the 943 CT exams included, 410 from a total of 403 subjects (146 male, ages ranging from 18 to 99 years) also had accompanying D-dimer values drawn within the specified time interval (43% of all PE protocol CTs). Of these exams, 30 (7.3%) were positive for acute PE (Table 1).

Table 1 Prevalence of acute PE in subjects with and without D-dimer values obtained.

D-dimer Data	D-dimer		Total
	Drawn	Not drawn	
PE CT Exams Performed (n)	410	533	943
Acute pulmonary embolus (n)	30	57	87
Acute pulmonary embolus prevalence (%)	7.3	10.7	9.2
95% confidence intervals	4.8 – 9.8	8.1 – 13.3	7.4 – 11.1

56 subjects underwent more than one PE protocol CT examination during 2007 (a total of 116 exams). Accompanying D-dimer values were drawn for 40 of these 116 exams. 18 of the 116 exams were positive for acute PE, and 5 subjects had acute PE on multiple exams. Only 1 follow-up PE protocol CT in which the initial exam demonstrated acute PE had an accompanying D-dimer value. In this particular case, the follow-up CT was negative for acute PE, and the accompanying D-dimer value was 0.22 μg/ml.

Of the 943 total exams, 117 of the reports described some limitation to the imaging. The type of limitation most cited was subject respiratory motion (96 exams). However, only 1 exam was deemed non-diagnostic (an exam that did not have an accompanying D-dimer value).

Prevalence stratified by D-dimer value

Using the findings on CT as the reference standard, the sensitivities, specificities, and positive and negative predictive values for using the stated D-dimer values are listed in Table 2.

As D-dimer values increase, the prevalence of acute PE also increases in a statistically significant manner ($p<0.05$, $\chi^2=55.07$,

Table 2 Acute pulmonary embolus at different D-dimer thresholds

Reliability	D-dimer Threshold Value (mg/ml)		
	1.0	2.0	4.0
Sensitivity (%; 95%CI)	96.7 (95.0 – 98.4)	80.0 (76.1 – 83.9)	46.7 (41.9 – 51.5)
Specificity (%; 95%CI)	56.6 (51.8 – 61.4)	78.4 (74.4 – 82.4)	93.9 (91.6 – 96.2)
Positive predictive value (%; 95%CI)	14.9 (11.5 – 18.3)	22.6 (18.6 – 26.6)	37.8 (33.1 – 42.5)
Negative predictive value (%; 95%CI)	99.5 (98.8 – 100.0)	98.0 (96.6 – 99.4)	95.7 (93.7 – 97.7)
Accuracy (%; 95%CI)	59.5 (54.7 – 64.3)	78.5 (74.5 – 82.5)	90.5 (87.7 – 93.3)

CI = Confidence interval.

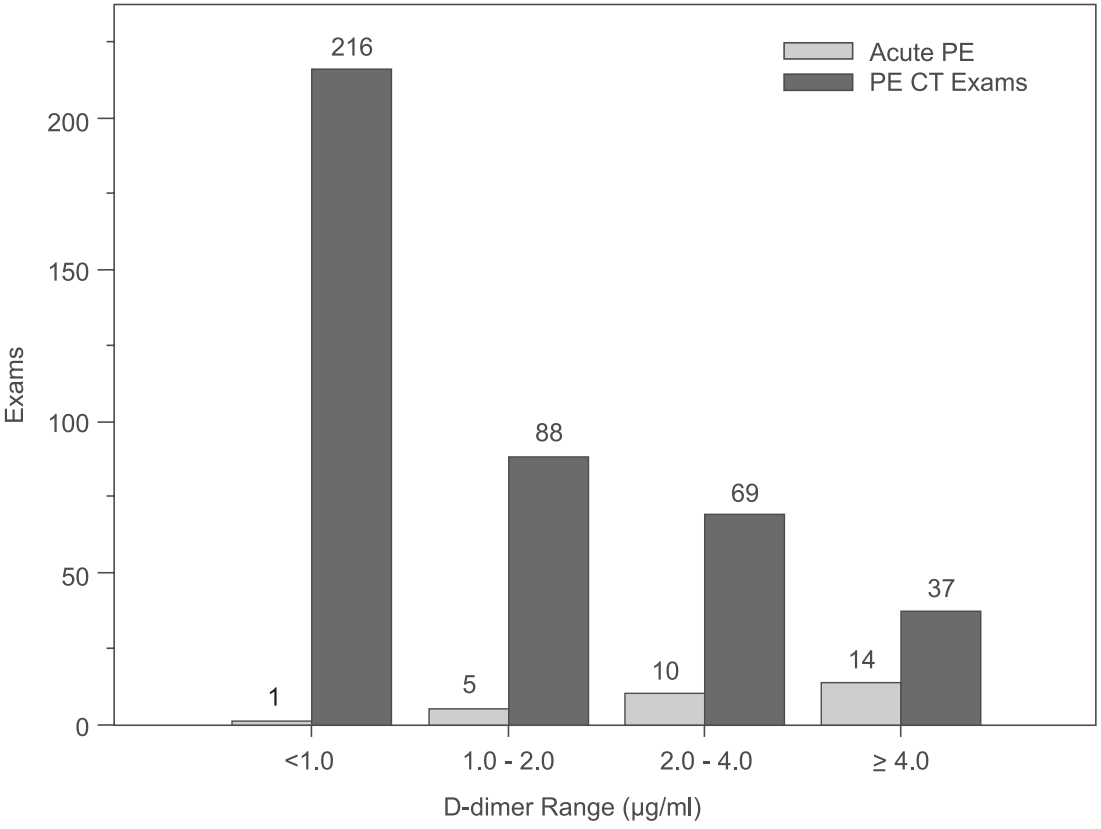

Figure 1 Distribution of acute PE studies according to D-dimer values for the 410 subjects in whom both D-dimer values and a PE protocol CT were obtained. Above each bar is the actual value from which the bar is generated (i.e., when D-dimer <1.0 µg/ml, there was 1 study positive for acute PE out of a total of 216 studies). Note that studies in which the D-dimer value equaled exactly 1.0 µg/ml are grouped in the "1.0-2.0" bin and those in which the D-dimer value equaled exactly 2.0 µg/ml are grouped in the "2.0-4.0" bin. See text for details.

3 degrees of freedom). Importantly, more than one-third of subjects with D-dimer values exceeding 4.0 μg/ml had acute PE (Figure 1).

Discussion

Our study retrospectively evaluated the relationship between the degree of D-dimer elevation and the prevalence of acute PE in low-to-intermediate risk hospitalized and ED patients. The overall prevalence of acute PE in this population is slightly higher than in those of other recent studies (5-7). A reason for this may be that, in contrast to these prior studies that evaluated only ED patients, our study also included PE protocol CT exams performed on inpatients, who may be at increased risk for PE relative to the ED population.

A well-documented shortcoming of the D-dimer assay in the investigation of acute PE has been its low specificity, as D-dimer values may be elevated in a number of other physiological and pathophysiological states (4). Our results suggest that as D-dimer values increase, they become much more specific for the presence of acute PE, reaching almost 94% specificity when D-dimer values exceed 4.0 μg/ml. This may be potentially helpful in patients who are unable to undergo a PE protocol CT because of decreased renal function or contrast allergy, though this requires validation in additional studies. Further evaluation in more subjects with markedly elevated D-dimer levels and their correlation with other measures of PE (i.e. nuclear medicine ventilation/perfusion scans and/or lower extremity Doppler ultrasound exams) would also be helpful in optimizing the management of such patients.

We note that the D-dimer threshold levels used in this study are significantly higher than the upper limit of normal, both employed by our institution (0.4 μg/ml) and recommended by the most comprehensive recent review (0.5 μg/ml) (4). The purpose of this study is not to evaluate the appropriate threshold for excluding acute PE, but to determine whether there is a relationship between the degree of D-dimer elevation and the prevalence of acute PE. However, it has not escaped our attention that acute PE prevalence in low-to-intermediate risk patients with lower D-dimer values is exceedingly rare (1 out of 216 in patients with D-dimer values <1.0 μg/ml). In fact, none of the subjects with D-dimer values <0.4 μg/ml (43 exams) or <0.5 μg/ml (80 exams) had acute PE; the one positive case for acute PE with D-dimer <1.0 μg/ml had a value of 0.62 μg/ml. Additional recent studies suggest that D-dimer threshold values higher than 0.5 μg/ml may safely exclude acute PE (7-9), which our data support.

Limitations

This is a retrospective study and is therefore subject to biases inherent in all such studies. D-dimer results would frequently have been available to the radiologist interpreting the CT at the time of its interpretation, so some element of bias in the interpretation is impossible to exclude. Patients at low-to-intermediate risk for acute PE that were unable to receive a PE protocol CT were not included in this study, and this may contribute to an additional selection bias. No correlation was made with the presence or absence of deep venous thrombosis as a possible explanation for an elevated D-dimer, though we reason that if the patients did not present with pulmonary and/or chest pain symptoms, they would not have been evaluated for acute PE using CT in the first place. There were 57 patients with acute PE who did not have D-dimer values drawn. However, if the clinical suspicion for acute PE is sufficiently great, checking a D-dimer value first would have been generally inappropriate if not unsafe (3, 5, 6, 10). However, having more cases of acute PE with correlating D-dimer values

would be helpful, particularly cases with markedly elevated D-dimer values. The decision to group ED patients and inpatients together in our study population was made largely due to the fact that many inpatients had some component of chest pain at their initial presentation to the ED. PE protocol CT exams were often ordered by admitting physicians while the patient was still in the ED or by the ED physician after the decision to admit had already been made, making appropriate segregation of these patients difficult. Additionally, different institutions use different D-dimer tests with different units (ELISA vs. latex turbidometric, plasma vs. whole blood, FEUs vs. D-dimer units, μg/ml, μg/l, ng/ml or mg/l), and caution must be used when comparing results and numerical values of D-dimer tests. And finally, while CT findings were used as the standard for the presence or absence of acute PE in this study, it is important to remember that despite being the current method of choice for diagnosis, PE protocol CT is not 100% sensitive for identification of all PEs; indeed, many prior studies report a sensitivity of <90%, particularly for smaller, more distal emboli (11).

Conclusion

The prevalence of acute PE by CT criteria in low-to-intermediate risk patients increases as plasma D-dimer values become increasingly elevated. In particular, markedly increased D-dimer values are highly specific for acute PE in this population, and such degrees of elevation may be helpful in the management of patients at risk for acute PE who cannot undergo PE protocol CT.

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Authors' contributions: Conception and design: AWB and NK; Acquisition, analysis and interpretation of data: AWB; Drafting the article AWB and GSJ, Revising it critically for important intellectual content: AWB, GSJ, and NK.

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Concentration of nitric oxide in patients' saliva from various metal restorative activities in the oral cavity

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Objective. The aim of this paper is to determine the concentration of nitric oxide (NO) in the saliva of subjects resulting from the presence of various metal restorative activities in the oral cavity. **Patients and methods**. The research was conducted on 20 subjects (12 women and 8 men), aged 18-30, with no metal fillings in their oral cavity and 20 subjects (11 women and 9 men) aged 18-30 with prosthetic and conservative work in their oral cavities made of various metals. The pH of the saliva was established by means of an "Orion" type pH meter at an optimal temperature of 25^0C. The concentration of NO was determined by conversion of NO_3^{-2} into NO_2^{-2} with the help of elementary zinc and then by colorimetric testing of the NO_2^{-2} concentration by means of the Griess reagent. **Results.** Our results established that the concentration of NO in patients with various metals (60.18±10.24) is 33.7% higher, which is statistically insignificant, in relation to the control group (45.01±6.28). **Conclusion.** Various metal restorative activities stemming from dentistry practice do not cause changes in the pH values of saliva and NO concentrations.

Key words: Saliva, Nitric oxide, Various metals.

Introduction

Nitric oxide (NO) acts as a biological messenger in the organism. NO is a short-lived gas that acts as a free radical since it contains an extra electron that enables high chemical reactivity (1). Recent evidence indicates the favourable anti-microbe effects of NO in the oral cavity (2). In the organism, NO acts as a physiological and pathophysiological mediator (3). The physiological roles of NO are numerous. NO participates in the vasodilatation of the soft muscles in the blood vessels, and inhibits the aggregation and adhesion of thrombocytes. It acts as a neuronal messenger and participates in the regulation of the immune response (4). NO plays an anti-bacteriolog-

ical role, inhibits the growth of bacteria and increases the macrophage cytotoxic mechanism. It easily penetrates the cell membrane, causes damage to bacteria, virus and tumour cells by means of mechanisms for the inhibition of the DNA synthesis (5).

The supragingival plaque, rich with anaerobe and aerobe flora, leads to a significant increase of oral NO (6). The current results support the thesis of NO as a modulator of bacterial proliferation and suggest that increased NO may contribute to a lower incidence of caries (7). Rehar et al. (6) have proved that the level of NO in the saliva of a patient with a generalised chronic periodontitis is increased and that NO can be regarded as a potential biological marker for detecting and monitoring the illness. The level of NO in the saliva of a smoker has been proven to be lower. The smoke components from a cigarette cause impairment of cells and reduce the cellular production of NO (8). There is clear evidence of lower concentrations of NO in patients who have used a wide spectrum of antibiotics in relation to those subjects who have not used antibiotics (9). NO is related to xerostomia, as the higher concentration of NO leads to the impairment of the salivary acinus. A change in the concentration of NO in saliva was noted in patients with diabetes mellitus, blood dyscrasia, autoimmune diseases and other diseases with xerostomia as one of the diseases symptoms. Dry mouth as a clinical symptom, with the consequent physiological, biochemical and immunological deficiency of the saliva's defensive function, contributes to an increased occurrence of periodontal and oral infections (10).

The pH values in the saliva affect the concentration of NO. Reductions of NO have been established in cases of decreased pH values (11). The oral cavity is moist and exposed to temperature changes, pH changes, and frequent changes of the chemical composition of the saliva, which favour the reaction of various metals in the mouth.

Various metals, with various electro-potentials, generate a galvanic cell through the saliva as electrolytes. The power of the micro-galvanic currents depends on the saliva or its compositions, pH, the surface voltage and the buffer capacity. The power of the galvanic cell also depends on the potential of the metal. If the difference in the potential is higher, the galvanic damage will be of higher intensity.

The aim of this paper is to determine the concentration of nitric oxide (NO) in the saliva of subjects resulting from the presence of various metal restorative activities in the oral cavity.

Patients and methods

The sample selection process was initiated on the grounds of the set aim. The research was conducted on 40 subjects divided into 2 groups. On the basis of subjective and objective indicators of their general health, none of the examinees had any manifestations of clinical or pathophysiological changes and were not taking medications which could affect the concentration of NO in the saliva. Control group - 20 subjects (12 women and 8 men), aged 18-30 years with no metal fillings in their oral cavity. Experimental group - 20 subjects (11 women and 9 men) aged 18-30 years with prosthetic and conservative work made of various metals.

The research was conducted on the grounds of a common approach to the examinees by analysing their medical history, an objective clinical examination and lab tests. After rinsing the mouth for one minute in order to remove debris and reduce the number of microorganisms, 2 ml of non-stimulated saliva was gathered into two sterile test tubes. The concentration of NO was determined by means of the conversion of NO_3^{-2} into NO_2^{-2} with the help of elementary zinc and then

by measuring the concentration of NO_2^{-2} by using the Griess reagent. After ten minutes of mixing in a vibrator at room temperature, the absorption of light (optical density) was measured by a spectrophotometer with a 545 nm filter. The concentration of nitrite was sensed from the standard curve with a known concentration of $NaNO_2$ (1.56 – 100nm). The distilled water, with a Griess reagent added, was used for a blind trial. The pH of the saliva was established by an "Orion" type pH meter at an optimal temperature of 25^0C. To measure the galvanic micro-current, a digital VOLTCRAFT VC 140 multi-meter was used.

Statistical analysis

The results were statistically processed and the mean value was established for each group, together with the standard deviation, the standard error of mean and values in percentage. A Student's t-test was used to determine the statistical significance of the mean values of the established parameters. The values $p<0.05$ were taken as significant.

Results

The results from analysing the values of the subjects' pH values in the saliva by groups are presented in Figure 1.

The results indicated that the mean value of the pH in the saliva of the subjects from the experimental group (6.89±0.09) was reduced by 1.43% in relation to the subjects from the control group (6,99±0,07) but this was not statistically significant.

The mean values of the pH in the saliva in the analysed groups by gender are presented in Figure 2.

The statistical analysis shows that no statistically significant difference by gender was established in the values of the pH of the saliva in the examined groups.

The mean values of the NO concentration in the saliva of the subjects from the analysed groups are presented in Figure 3.

The results established that the mean values of NO in patients with various metals in their oral cavity (60.18±10.24) are 33.7% higher, which is however statistically insignificant, in relation to the control group (45.01±6.28).

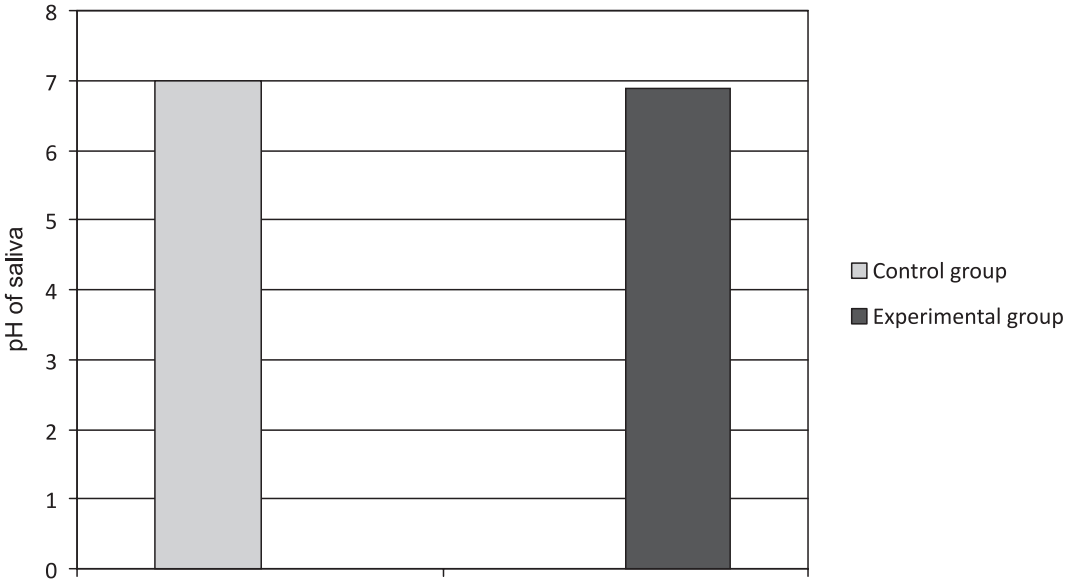

Figure 1 Mean values of the subjects' pH in saliva by groups

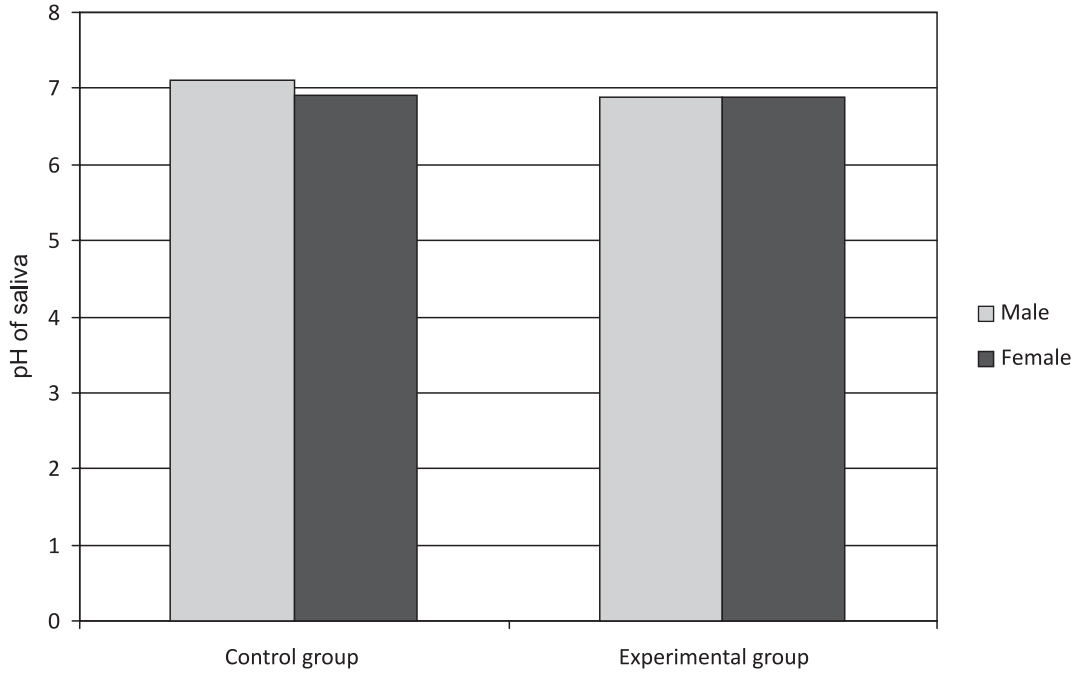

Figure 2 Gender differences in the mean values of the pH in the saliva of the subjects

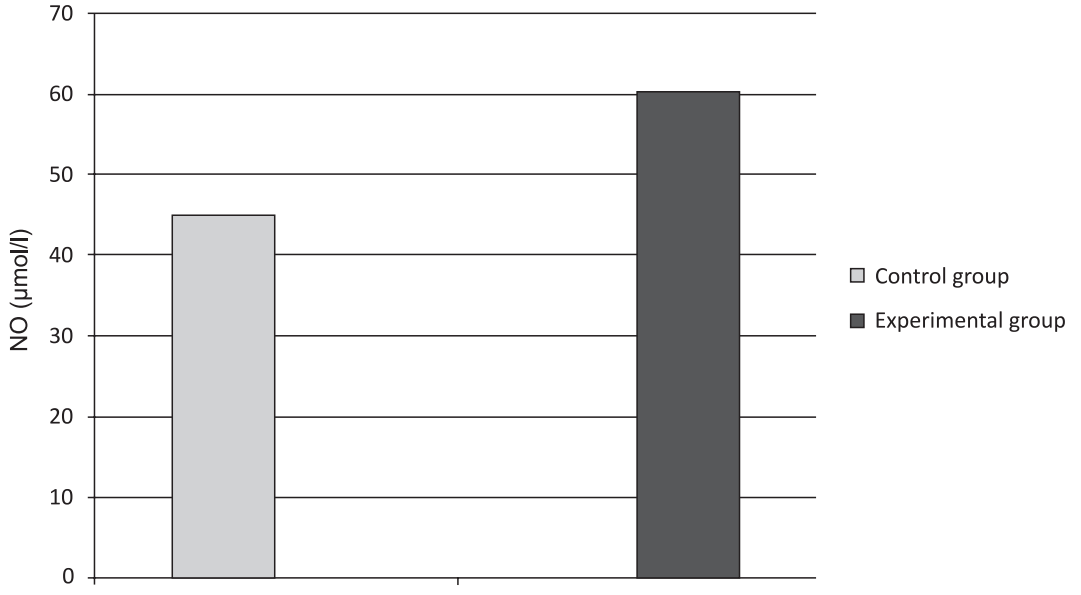

Figure 3 Mean values of NO concentration in the saliva of the subjects

The mean values of the NO concentration in the saliva of the subjects distinguished by gender are presented in Figure 4.

The statistical analysis shows that no statistically significant difference by gender was established in the concentration of NO in the saliva in the examined groups.

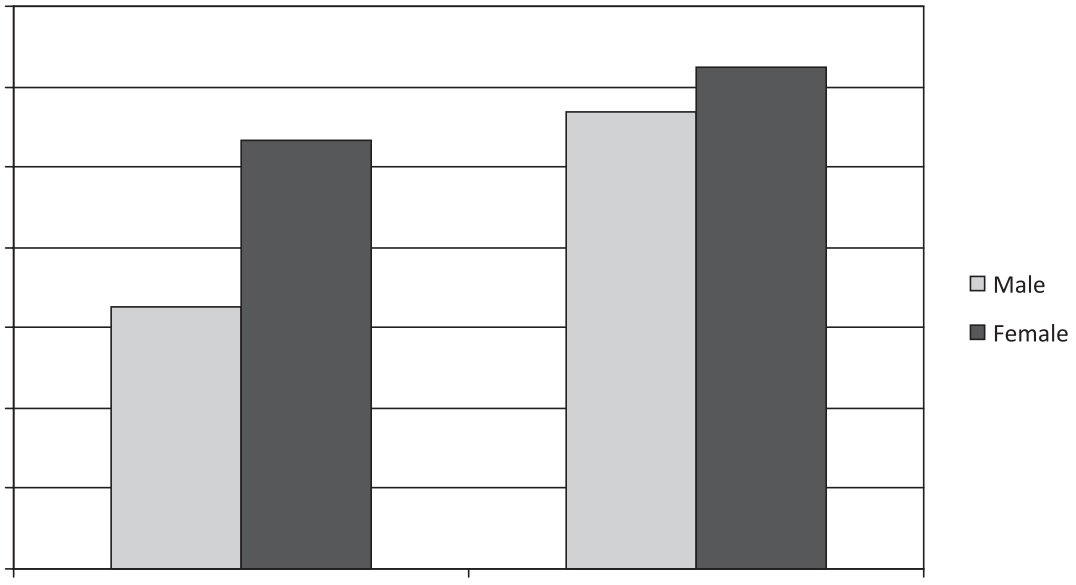

Figure 4 Gender differences in the mean values of the concentration of NO in the saliva of the subjects

Discussion

NO is a topic of interest amongst various scientific groups in view of the fact that it performs various physiological and pathophysiological processes in the organism. Many authors have confirmed the effects of NO on the vasodilatation of the cerebral, coronary, pulmonary, renal and musculoskeletal circulation (1, 3, 12). NO enters the saliva through nerve fibres, capillary endothelium and macrophage and can also be synthesised in the salivary gland acinus. The NO modifies the concentration of Ca^{+2} in the salivary cell where it is synthesised and moves onto the surrounding tissues through simple diffusion (13). NO plays an anti-bacteriological role, inhibits the growth of bacteria and increases the macrophage cytotoxic mechanism. As a strong reactive radical, NO participates in the non-specific defensive mechanisms of the oral cavity, which is linked to the significant immunological potential of NO in the saliva of our subjects.

Our research has shown that the values of the NO concentration in the saliva of the subjects with various metal restorative work are higher than in those in the control group but this is not statistically significant. The difference between the values of NO in the subjects' saliva distinguished by gender is not statistically significant.

Our results regarding the values of NO concentration in the saliva of the subjects with various metal restorative work in their oral cavity can be justified by the small sample (20 subjects) and the young age group (18-30 years old) of the examinees, who did not have systemic diseases and pathophysiological changes in their oral cavities.

In our research, the pH values in the saliva of the subjects from both the control and the experimental group were neutral. The subjects did not have systemic diseases, periodontal inflammation or salivary secretion disorders, which would affect the concentration of NO and the pH values of saliva (10).

The concentration of NO in our research was higher in the middle of the neutral pH value of the saliva, which corresponds to the research conducted by Palmerini (11) who attested that the concentration of NO is

higher in the middle of the neutral pH values of saliva.

Differences by gender in the pH values of saliva in the experimental and control groups were not statistically significant.

The pH value of saliva is important for the release of ions originating from various metal prosthetic and conservative work. Ankea et al. (14) have verified that the release of ions of cobalt, chrome, iron, nickel and zinc from analysed alloys depended on the time of the alloy exposition to phosphate buffer pH 6.0. This data is important since cobalt-chrome-molybdenum alloy, which is most commonly used as material for making skeletal prosthesis, has been found in the oral cavity of patients for very many years.

Moreover, Wataha (15) claims that the period of the presence of dental alloys in the oral cavity is an important factor in the intensity of ion release. In our research, various metal restorative work did not cause changes in the pH values of the saliva.

Karov et al. (16) indicate that the galvanised micro-current can cause prickling, burning, paraesthesia and pain. Baričević et al. (17) have shown in their paper that burning mouth syndrome was more frequent in patients with dental metals but there was no correlation between high concentrations of nickel and chrome in the saliva and burning mouth syndrome.

We did not verify subjective symptoms of prickling, higher or lower intensity of burning and metallic taste in our patients with various metal restorative work in their oral cavities, which is not in compliance with the research conducted by Karov (16).

The subjects from the experimental group had had their various metal prosthetic and conservative work for one year and we thus deem that this short time period was insufficient for any consequences caused by oral galvanism.

Our results cannot be compared with the results of other researchers since we have not come across data on the physiological and immunological mechanisms of NO in the saliva in the presence of various metal restorative work in the oral cavity.

Considering that we have only examined the presence of various metals, we believe that it would be interesting to explore the level of NO in the saliva compared to the number of various conservative and prosthetic metal solutions (metal fillings, crowns, and mobile prosthetics), and the time they are present in the oral cavity.

Technological procedures have, so far, not confirmed an alloy which would be completely stable in the organism, and which would show no signs of biodegradation.

In stomatology it is recommendable to use alloys with a known composition and good biocompatibility. Precise technological procedures in the laboratory are an important precondition for the prosthetic treatment of patients.

The role and the importance of NO and its metabolites, as well as the effect of various factors on the concentration of NO in the saliva of patients with various metal prosthetic and conservative works are dilemmas which can be used as subjects of further research.

Conclusion

The values of the NO concentration in the saliva of the examinees with various conservative and prosthetic metals in their oral cavity are higher but the difference is is not statistically significant in relation to the control group. The variations in the mean value of the NO concentration in the saliva of examinees distinguished by gender do not have statistical significance in either group. The various metal restorative works in our patients have not affected the changes to the pH values of the saliva.

Conflict of interest: The authors declare that they have no conflict of interest. This study was not sponsored by any external organisation.

Authors' contributions: Conception and design: EP and JH; Acquisition, analysis and interpretation of data: JH, EP and AD; Drafting the article: EP and SH, Revising it critically for important intellectual content: EP, JH and AD.

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Review article

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Use of computer assisted orthopedic surgery in pelvic and acetabular trauma

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Computer assisted orthopedic surgery (CAOS) is a recent concept in orthopedics. Its use in orthopedic trauma is becoming more popular. Pelvic and acetabular trauma is one of the applications where CAOS can play an important role to facilitate the surgery. In this review article, we provide an overview of the structure of CAOS with special emphasis on its role in pelvic and acetabular trauma. The use of CAOS has many advantages in the field of orthopedic trauma, however, many obstacles are still present that prevent its wide use.

Key words: Pelvis, Fractures, Osteosynthesis, Computer navigation.

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Introduction

Computer assisted orthopedic surgery (CAOS) is a new concept in orthopedic surgery. In orthopedics, CAOS has been most extensively used in arthroplasty (1-7) and to a less extent in spine surgery (8, 9). CAOS in arthroplasty allows for defining patient anatomy (e.g. acetabular abduction and flexion, hip offset and length) and recreating it more precisely and with less outliers than conventional methods of reconstruction (1-7). CAOS in spine surgery allows for more accurate insertion of pedicle screws, especially in cases of spine deformity and scoliosis, in which the normal anatomy of the spine is altered and finding the pathway of the pedicle screw becomes more difficult (8, 9). Recently, there has been a growing interest in using CAOS in orthopedic trauma (10). In 1998, the National Institute of Health/American Association of Orthopedic Surgeons Workgroup on Technology Transfer in Orthopedics suggested that orthopedic trauma may become one of the fields where image-guided surgery might have an important role (11).

In orthopedic trauma, CAOS has been introduced to improve the accuracy of placement of the implants in

treating pelvis and acetabular fractures. The complex anatomy of this region requires significant use of fluoroscopy to guide the insertion of the implants, i.e. screws.

The components of CAOS

A CAOS navigation system consists of a computerized navigation unit (which has the procedure-specific software) connected to an optical tracking camera; trackers (light emitting diode - LED) which are reference guides attached to the surgical site, LED attached to the image source (C-arm), LED attached to the surgical instruments ("smart") and a monitor (12, 13) (Figure 1). The process of "image guided navigation" has three elements:

Data acquisition images of the patient anatomy / pathology (fracture) are entered into the computer and these images are used to guide the surgeon to perform the intended procedure. In trauma surgery, data acquisition is usually performed by one of three imaging sources (conventional 2D fluoroscopy, 3D fluoroscopy, CT scans, sees below).

Registration the process of relating the collected data of the patient's three-dimensional anatomy / fracture (from the data acquisition step) to the patient's actual position and anatomy on the surgical field. This is done by the computerized navigation unit identifying the LED tracker attached to the patient's body. In cases of pelvic and acetabular trauma, the LED trackers are usually inserted into the iliac crest. Surgical tools with a tracker attached to them are also registered by the navigational system.

Tracking provides feedback during surgery by identifying the relative position of the LED attached to the bone and surgical tools. The navigation unit tracks the position of the surgical tools in relation to the patient's anatomy and the surgical instruments are displayed on the monitor, superimposed on the patient imaging study, as

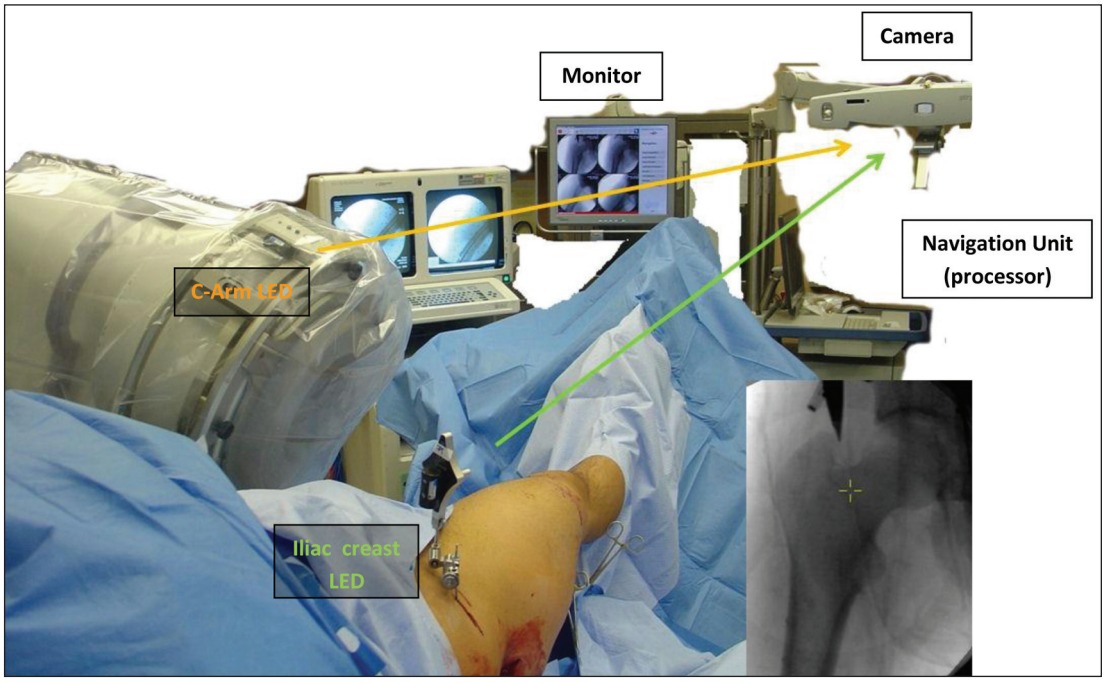

Figure 1 Composition of CAOS: LED trackers attached to the iliac crest, LED attached to C-arm, optical tracking camera, computerized navigation unit, monitor

continuous virtual real time navigation. This will give feedback to the surgeon about the relative position of his instrument or his implant. The surgeon can use this feedback to adjust the position of the instrument or the implant relative to the patient's anatomy, to follow the correct anatomical pathway (12). There are mainly three types of data acquisition in trauma surgery:

• **Intra-operative Conventional 2D Fluoroscopy** Two-D (dimensional) fluoroscopy is the most popularly used method of data collection in trauma surgery, including pelvic and acetabular trauma. The C-arm is widely available in the majority of hospitals, and the amount of radiation generated by the C-arm is less than CT scans (computer tomogram) and 3D fluoroscopy (see below). The disadvantage of conventional 2D fluoroscopy lies in the decreased delineation of anatomic structures, especially in anatomically complex regions (e.g. the pelvis and acetabulum).

• **Intra-operative 3D Fluoroscopy** This new technology allows 3D reconstruction based on multiple intra-operative fluoroscopy images. During 3D fluoroscopy there is increased patient exposure to ionizing radiation, with additional exposure(s) if the operating field has to be broadened or if the reduction has to be checked or repeated. With the Iso-C® (Siemens, Germany) device, a multi-planar reconstruction for each field (12 x 12 x 12 cm) is calculated from 100 C-arm images.). 3D has improved image quality compared to 2D fluoroscopes and better availability compared to CT. The use of 3D fluoroscopy based navigation is becoming more popular nowadays (e.g. acetabular and calcaneal surgeries) (14, 15).

• **Computerized Tomography** Computerized tomography is used for precise fracture delineation in spine, acetabulum or pelvic operations. It has the advantage of high accuracy for anatomy identification. There are two types of CT acquisition: pre-operative CT and intra operative CT.

– *Pre operative CT* The major disadvantage of using pre operative CT in trauma surgery is the necessity for absolute fracture stability, so the relationship between the bone fragments may be sustained throughout the period from the preoperative CT acquisition to the time of surgery, i.e. stable pelvic or acetabular fractures (16, 17). The matching procedure (registration) in pelvic and acetabular trauma can be difficult, because the pelvis is surrounded by abundant soft tissue (14).
– *Intra-operative CT* This eliminates the disadvantage of multiple imaging for registration because registration can be done at the same time as data acquisition. Another advantage of intra-operative CT is that it decreases the risk of displacement of the fracture between the time of CT acquisition and surgery. Intra operative CT is more commonly used in percutaneous sacroiliac fixation or acetabular fixation (18, 19). However, it requires performing the procedure in the CT suite or having a CT machine in the operating theatre. It is an expensive option, not available in many places, and irradiation of the patient is higher compared with C-arm usage.

Sacral and iliosacral screw insertion

Most sacral fractures and iliosacral joint disruptions are now being treated by closed reduction and percutaneous screw fixation. Due to the very narrow safe corridor for these screws, it is necessary to ensure the correct pathway for the screw. Guide wires are inserted first and then after checking that these wires are in the desired place, cannulated screws are threaded over the guide wires. To obtain the correct pathway, the guide wires have to be in the correct di-

rection in three views of the sacrum (inlet, outlet and lateral). Using conventional fluoroscopy for insertion of these screws is not a simple task. After it is done in one plane, the C-arm needs to be moved to the other planes to check the position of the implant. The C-arm has to continue shifting between the three positions (a large amount of irradiation for the patient and the surgeon) throughout the whole procedure. Multiple attempts are usually required before achieving the correct pathway as the surgeon can only assess the pathway in one plane at a time. The perfect trajectory may be obtained in one view and when the other view is checked, the guide wire may be in an incorrect position. Also with false passages, the guide wire may encroach over the nerve foramens and cause nerve palsy or vascular damage (20-22). To improve precision, decrease the radiation, and shorten the surgery time CAOS should be used when inserting sacral and iliosacral screws.

Stöckle et al. (14) inserted 28 iliosacral screws (27 in S1 and 1 in S2) by image guided navigation. The authors had 27 of 28 screws (96%) well placed as assessed by postoperative radiographs and CT scan (14). In another report, CAOS was associated with accurate placement of 21 out of a total of 22 implanted iliosacral screws in 10 patients (9 patients with trauma and one with instability), where one screw perforated the anterior wall of the sacrum and had to be revised; this deviation of the screw was attributed to the bending of the navigated guide during implantation. The authors recommended using a navigated sleeve rather than a navigated power driver, as this may lead to less bending of the guide pin (19). Schep et al. (23) treated 24 patients with post partum pelvic pain syndrome. Each patient had 2 screws inserted bilaterally (96 screws). They compared 48 iliosacral screws inserted with fluoroscopy based navigation with 48 screws inserted by conventional fluoroscopy. The fluoroscopy time in the navigation group was 0.7 minutes versus 1.8 minutes in the conventionally treated group. There was one case in the navigation group that developed a post-operative S2 sensory deficit (23). It is important to note that their study was performed on patients with no fractures. In cases of fractures, it is mandatory that the fracture parts do not move after loading the fluoroscopic images into the navigation system. If the fracture site has moved after reduction (which is not common with sacral fractures), the virtual pictures on the navigation monitor will not represent the actual reality and registration must be repeated. Other authors have also used navigation for insertion of sacroiliac screws with similar results (24, 25).

Acetabular and pelvic fixation

Percutaneous fixation of the posterior and anterior column of the acetabulum has become more popular recently (21, 26). Multiple fluoroscopy views (iliac, obturatory, inlet, outlet, iliac-inlet view and obturator-outlet views) are needed to obtain the correct pathway and avoid complications (e.g. penetration of the joint space, neuro-vascular injury) and secure fixation (optimal screw orientation and screw length).

To date there have been no studies comparing the use of conventional fluoroscopy with CAOS in inserting percutaneous anterior and posterior column screws. Rosenberg et al. (18) used image guided navigation in inserting eight percutaneous screws in five patients with minimally displaced acetabular fractures and the result was optimal placement of 6 out of 8 screws; the bending of the guide wire was considered responsible for the 2 incorrect screw placements. Crowl and Kahler (2002) reported the use of image-guided navigation in performing minimally invasive fixation of 9 anterior column fractures of the acetabulum and this resulted

in safe placement of all screws (27). Image guided navigation in acetabular fractures has been described by other studies (level III or IV) with similar good results (14, 24, 25).

Our method of applying CAOS in pelvic and acetabular fracture

After positioning the patient on the table, closed reduction is obtained (or maintained) using traction and manipulation. The trackers (LED) are attached to the iliac wing bone. Appropriate radiographs are obtained after achieving the closed reduction. For sacral, sacroiliac and pelvic injuries: anteroposterior pelvis, lateral sacral, inlet and outlet views are necessary. Acetabular injuries require: anteroposterior pelvis, obturatory and iliac views. For percutaneous anterior or posterior column screws: we add the obturator-outlet view and iliac-inlet view for better assessment of the pathway of the guide wire and ensure that it does not penetrate the hip joint or violate the cortex (28).

Those images are related by software to the patient's anatomy using a reference (patient tracker attached to iliac wing). The instruments (e.g. guide sleeve) are registered. The C-arm machine is moved away from the operating table and we operate observing the monitor of the navigation unit, which shows the four saved and needed views of the patient's anatomy.

The pathway for the guide wire of the cannulated screw (which is controlled by the registered sleeve guide) will be visible on the screen in relationship with each of four needed images (virtual reality). This allows us to continuously check the position of our guide wire. There is no need to repeat the radiographs or move the C-arm from one position to the other. The software has the option of extending the trajectory from the bone surface towards the desired position calculating the length and thickness of the screw. Final radiographs are taken at the end of the procedure to ensure that the virtual reality on the monitor is identical to the actual position of the screw (Figure 2).

Advantages of using navigation in pelvic and acetabular trauma

The reduction of radiation exposure (compared to the use of conventional fluoros-

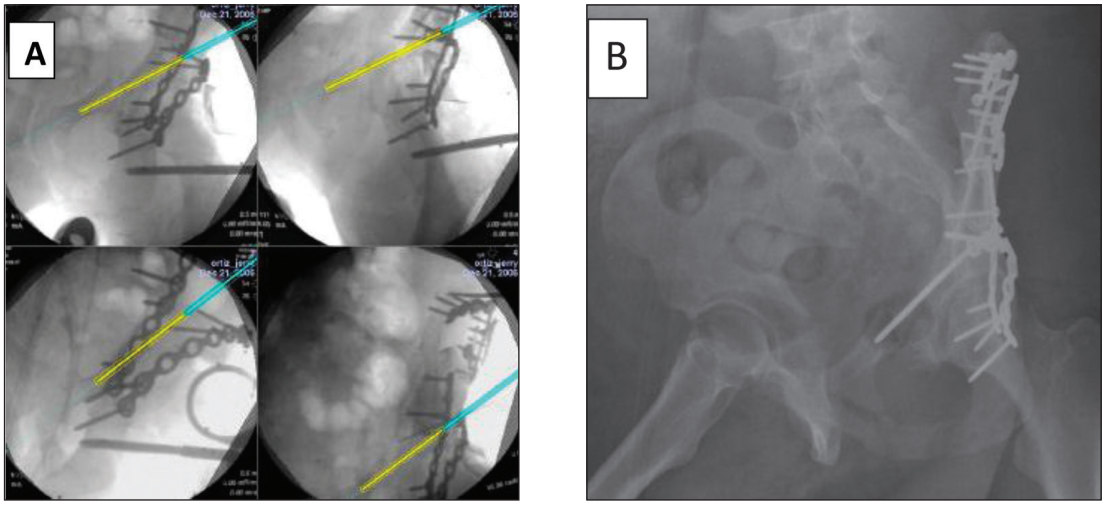

Figure 2 After fixing the acetabular fracture from the posterior approach, CAOS is used to insert the percutaneous anterior column screw. (A) Four views are shown on the monitor of the navigation unit with a virtual image of the guide wire trajectory on these views. (B) Final radiograph

copy) is one of the main advantages of using the CAOS. CAOS can show the surgeon continuous virtual reality images of the instruments and implants, with no need to repeat the fluoroscopy.

With image guided navigation, radiation exposure can be up to one fourth of radiation with conventional radiology (29). Also the initial radiographs (mounting radiographs for the navigation unit) can be taken while both the surgeon and the operating room personnel are standing away from the source of radiation. After that, the source of radiation can be removed and the surgeon and his assistants can come close to the patient with no extra radiation needed. It is known that the radiation-induced cancer risk is cumulative, what places surgical staff under real risk (13, 19, 24, 30-37).

Also, CAOS decreases the number of false passages. In conventional fluoroscopy, the guide wire is moved forward while being viewed in one plane and then its position is checked in the other needed plans with the C-arm. If the direction of the guide wire is incorrect, it will create a false passage. With CAOS, the procedure is done with the imaginary guide wire (trajectory) in all four planes on the navigation monitor before advancing the guide wire, assuring that the guide wire is in the correct corridor before drilling it.

There are certain obstacles to widespread use of navigation in trauma practice

It can be used only in fractures that are stable and where the bone ends do not move in relation to each other (if it is to be used in an unstable fracture, both fracture ends have to be traced by trackers). Again, bending of the guide wire may be responsible for disparity between the real position of tip of the guide wire and what is shown on the monitor of the navigation. This has been described as a possible cause of the inaccurate position of screws inserted by navigation in pelvic trauma surgery (18, 19). To avoid this problem, use of thicker wire or navigating solid drills (and using solid non-cannulated screws), rather than guide wires, is recommended.

There is a learning curve for using navigation in trauma. The position of the trackers should be where the pathways between them and the navigation unit is not blocked by the C arm, the surgeon, surgical instruments or (with obese patients) abdominal folds. Each time the pathway between the trackers and the navigation unit is interrupted, the picture on the computer screen will disappear, which may cause some frustration and delay. Navigation requires extra steps for set up in the operating room (insertion of tracking and registration), which will increase the surgery time at first when using this technology. With repeated use of navigation, the surgeon and operating room personnel may become more acquainted with these steps and the set up of navigation may cause a minimal increase in surgery time.

CAOS increases the cost of surgery. This may be the main obstacle against widespread use of navigation. There is a significant cost associated with CAOS, the initial hard- and software cost between 200,000 - 250,000 US dollars, followed by the cost of maintenance and software updating.

Conclusion

CAOS is becoming more popular in orthopedic trauma surgery, especially pelvic and acetabular fixation because of their complex anatomical nature and the need for extensive use of radiographs. It has positive effects that will benefit both patients and surgeons. It is an emerging technology and its use will expand tremendously with time, despite the current limitations. Orthopedic surgeons should be aware of CAOS and its development as it is expected to become more user-friendly and more affordable in future.

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Review article

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Vitiligo and other hypopigmentation disorders in children and adolescents

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The loss of pigment, either partial (hypopigmentation) or complete (depigmentation), can have a major psychological impact on patients. Hypopigmentation disorders, congenital and acquired, are very numerous, and many of them are rarely seen. This paper provides an overview of the most common hypopigmentation disorders in children and adolescents, stressing the importance of vitiligo and autoimmune disorders in patients. Vitiligo is an acquired disease, possibly of autoimmune nature, sometimes with a clear hereditary component, which is characterized by progressive, clearly defined, milky white spots on the skin and/or mucous membranes. In about 50% of patients vitiligo occurs before 20 years of age. The clinical picture of vitiligo in children and adolescents is similar to adults, but there are some differences in the epidemiology, their associations with other endocrine and/or autoimmune diseases and treatment of vitiligo in children compared to adult patients.

Key words: Hypopigmentation, Vitiligo, Child, Adolescent, Diagnosis.

Introduction

Pigmentation disorders can be congenital or acquired. Congenital disorders of pigmentation, often result from mutations of many genes that govern the processes of development of melanoblasts, their migratory movements towards the periphery, and their differentiation into mature melanocytes that produce pigment. The acquired pigmentation disorders are mainly due to qualitative or quantitative defects of melanocytes (e.g. vitiligo) in the skin and mucous membrane (1, 2).

The loss of pigment, either partial (hypopigmentation) or complete (depigmentation), can have a major psychological impact on patients. In many religious writings, depigmentation is described as a sign of dangerous, communicable diseases (3). In ancient times, vitiligo was equated with leprosy, in which hypopigmentation also occurs, and these patients were varying degrees of social

outcasts (1, 2, 4). Although it is now known that most hypopigmented changes in the skin are not contagious and dangerous, loss of pigment causes psychological problems for a large number of patients (5). Many skin diseases are accompanied by hypopigmentation or depigmentation. Vitiligo, pityriasis alba and pityriasis versicolor and varietas alba are seen in everyday clinical practice, and these diseases will be discussed (6).

Vitiligo

Vitiligo is an acquired disease, possibly autoimmune by nature, sometimes with a clear hereditary component, which is characterized by progressive, clearly circumscribed, milky white spots on the skin and/ or mucous membranes. The name vitiligo comes from the Latin *vitium* (anomaly) or *vitelius* (white patches on calf's fur) (1, 2, 3).

Epidemiology

Vitiligo occurs among people of all races and climates. It is believed that 1-2% of the world's population is affected. It can occur in all age groups, but in half the patients it occurs before 20 years of age (1, 2, 3, 4, 7). Some authors suggest that 25% of patients first develop the symptoms before the age of eight, and 50% of patients prior to 10 years (7, 8, 9).

The epidemiology of vitiligo in children is similar to adults, but there are certain characteristics of children's vitiligo. Unlike adults, where the frequency of vitiligo in both sexes is about the same, vitiligo in children is more common in girls (10). A family history of associated endocrine and / or autoimmune disease is more common in children with vitiligo than in adult patients. Vitiligo is present in the family history of 12% to 35% of affected children, as opposed to about 8% of adult patients (7, 10, 11, 12).

The etiology and pathogenesis of vitiligo

The etiology and pathogenesis of vitiligo is not fully explained. It is known that genetic factors play a role in the development of vitiligo: a positive family history in up to 30% of patients, described cases of vitiligo in twins, and the fact that vitiligo occurs with diseases that have a clear genetic basis, such as diabetes mellitus, suggests that role (3, 4, 13). Recent research suggests two possible modes of inheritance of vitiligo, which are associated with the age at which vitiligo appeared (14). In patients with early onset of vitiligo (before age 30), vitiligo is caused by the dominant mode of inheritance with incomplete penetration. However, in patients with late onset (after age 30), predisposition to vitiligo is the result of a recessive genotype and the influence of the external environment (15, 16). An earlier start of vitiligo (up to 7 years) was found in children with vitiligo and a positive family history of vitiligo (17). There is clear evidence of the association of certain MHC haplotypes with a positive family history of vitiligo, onset timing, severity of disease and ethnic origin (15, 16). As a possible theory for the etiology of vitiligo the following are cited: the autoimmune, oxidative stress theory (theory of self-destruction of melanocytes) and the neurogenic theory (1, 2, 3, 4).

The autoimmune theory is supported by experimental evidence and clinical association with other autoimmune diseases: pernicious anemia, *Addison*'s disease, type 1 diabetes mellitus (T1DM), juvenile rheumatoid arthritis, alopecia areata and especially *Hashimoto*'s thyroiditis (1, 3, 18, 19, 20, 21). Vitiligo is relatively common in of autoimmune polyglandular insufficiency syndrome (APS), especially in type I (APS-I), where 25% of patients have vitiligo (22). Patients with vitiligo often have organ-specific serum autoantibodies, especially antithyroid

(antithyroglobulin and antibodies against thyreoperoxidase) and antiparietal autoantibodies. Some research suggests that an increased incidence of autoimmune thyroiditis in patients with vitiligo is genetically determined. The locus on chromosome 1 - AIS1 (*engl. autoimmunity susceptibility*), responsible for the tendency to autoimmune responses, particularly for vitiligo, in the presence of other genes (e.g. the main histocompatibility complex localized on the short arm of chromosome 6), combined with exposure to external or internal factors, may mediate in the development of *Hashimoto*'s thyroiditis in patients whose AIS1 is susceptible (23, 24). It is known that genes on chromosome 17p13 contribute to the development of certain autoimmune diseases, which include generalized vitiligo, autoimmune thyroiditis, T1DM, rheumatoid arthritis, psoriasis, pernicious anemia, systemic lupus erythematosus, and *Addison*'s disease. Recent studies describe the NALP1 protein as a gene that regulates the intactness of the immune system. Changes in the DNA sequence in the NALP1 domain, are associated with an increased risk of generalized vitiligo and/or other joint autoimmune diseases, such as autoimmune thyroiditis (25). It is believed that autoimmune mechanisms play a key role in the pathogenesis of non- segmental vitiligo (4).

The melanocytes of active vitiligo reveal an increase in oxidants and antioxidant enzyme deficit, with resultant oxidative damage to melanocytes (3, 26, 27). The emergence of segmental vitiligo can be best explained by the neurogenic theory according to which the nerve endings release the neurochemical mediators that inhibit melanogenesis or have toxic effects on melanocytes, destroying them. Microscopic examination and ultrastructural investigations in people with segmental vitiligo showed damage to axons and disrupted neuropeptide balance in vitiligo lesions (27, 28). As possible provoking factors for the occurrence of vitiligo the following are cited: stress, sun burns, recurrent skin injuries. It is believed that different mechanisms may cause the same phenotype: vitiligo is a heterogeneous disease in terms of etiology (2, 4).

Clinical characteristics of vitiligo

The main change in vitiligo is depigmented macula, usually round or oval-shaped, milky white color, with a diameter of several millimeters to several centimeters (1, 2, 3, 4). The skin surface in vitiligo is smooth. Atypical maculae (artificial) appear at the site of injury, lacerations, friction and burns and are the result of *Köebner*'s phenomenon (1). Vit-

Table 1 Characteristics of non-segmental and segmental vitiligo

Characteristics	Non-segmental vitiligo	Segmental vitiligo
Prevalence (%)	72-95	5-8
Distribution pattern	Symmetrical, not limited to the dermatome	Unilateral, limited to the dermatome
Onset	Any age	Young age
Course	Variable, often new lesions occurring during life (progressive course)	Initial rapid progression, then the activity of lesions limited to the period up to 2 years
Köebner's phenomenon	Often present	Rarely present
Association with autoimmune diseases	Frequent	Extremely rare
Etiology	Probably autoimmune	likely neurochemical

iligo can occur anywhere on the skin. Predilection sites are around the natural openings - the eyes, mouth, nipples, navel and genitals. In the majority of patients vitiligo lesions first appear on skin exposed to the sun. In vitiligo patients polyosis commonly occurs ("white lock of hair") and halo nevus (*Sutton* nevus) (3).

Vitiligo can be divided into the non-segmental and segmental forms of the disease (1, 2, 4, 9). In segmental vitiligo depigmented plaques are limited to a dermatome. The characteristics of these two types of vitiligo are given in Table 1. In clinical practice the generally accepted classification of vitiligo is by *Kovacs* (3). According to this classification, vitiligo is divided into focal, segmental, generalized, acrofacial and universal types, which differ not only morphologically but also in their natural course, response to treatment and prognosis (1, 2, 3).

The association of vitiligo with other endocrine and / or autoimmune diseases

Adult patients with vitiligo, especially with the generalized form of the disease, are at increased risk of developing a number of endocrine and / or autoimmune diseases, including thyroid disease, T1DM, pernicious anemia, *Addison*'s disease, rheumatoid arthritis and alopecia areata. Vitiligo can occur before, concurrently or after the occurrence of one or more endocrine and / or autoimmune diseases (1, 3). Epidemiological studies, carried out on a large number of children with vitiligo, showed no increased risk of contracting one of these endocrine and / or autoimmune diseases (10, 29, 30, 31, 32).

Patients with vitiligo most commonly suffer from thyroid disease (30-40%), precisely *Hashimoto*'s thyroiditis, then hyperthyroidism, hypothyroidism and *Graves-Basedow*'s disease (1, 33, 34, 35). Unlike adults, children and adolescents with vitiligo demonstrate increased frequency of exclusively *Hashimoto*'s thyroiditis. Considering the fact that vitiligo usually precedes autoimmune thyroiditis, a possible early diagnosis of the latter is possible. It is therefore recommended that children and adolescents with non-segmental vitiligo undergo annual screening for antibodies against thyreoperoxidase (TPO-Ab), antithyroglobulin antibodies (Tg-Ab) and thyroid-stimulating hormone (TSH) (36, 37, 38).

Therapy

Treatment of vitiligo should be initiated with a consultant dermatologist, after presenting therapeutic possibilities and their effectiveness to the patient. The choice of therapeutic options depends primarily on the age and type of vitiligo patients. In all cases it is necessary to use measures to protect against solar radiation and use photoprotective methods (1, 2, 39, 40).

- Phototherapy. There are two basic forms of generalized vitiligo phototherapy: PUVA (*Psoralen* + UVA) photochemotherapy using photosensitive drug (8-metoxypsoralen) in combination with UVA radiation (320-400 nm wavelength) and UVB rays of narrow range of wavelengths of 311 nm (*Narrow -band UVB*). Targeted laser phototherapy is recommended for focal vitiligo (2, 41, 42).
- Local corticosteroid therapy: treatment for at least 3 to 4 months (watch for side effects of corticosteroids!) (7).
- Other forms of local therapy (melagenin, calcineurin-inhibitors / pimecrolimus, tacrolimus /, calcipotriol, pseudocatalase) (43, 44, 45)
- Depigmentation (monobenzyl hydroquinone ether and 4-methoxy-phenol as a cream) with extensive and generalized vitiligo only in adults and adolescents (2).

– Surgical treatment (epidermal grafting obtained by suction, autologous skin graft method, mini-transplantations) is the method of choice for segmental vitiligo in adults and adolescents (46).
– Cosmetic camouflage methods (47).

The differential diagnosis of vitiligo

The diagnosis of vitiligo is set on the basis of history and the characteristic clinical picture, additional laboratory analysis are rarely needed, skin biopsy even rarer. In children there may be differential diagnostic difficulties in relation to other diseases that are accompanied by hypopigmentation, such as pityriasis alba, pityriasis versicolor varietas alba, post-inflammatory hypopigmentation, piebaldism, morfea, leprosy, tuberous sclerosis, naevus depigmentosus and lichen sclerosus et atrophicus (4, 6). A special difficulty for the recognition of vitiligo arises in fair-skinned people, where it is harder to spot hypopigmentation or depigmentation. A *Wood*'s lamp (emitting UV rays with wavelengths of 320-400 nm, with a maximum of 365 nm), makes it possible to spot the differences between the complete loss of pigment in vitiligo and various other diseases accompanied by hypopigmentation. Knowing the type of inheritance, the characteristic clinical patterns and symptoms of these diseases is necessary to set an accurate diagnosis of vitiligo (4, 6, 11).

Pityriasis alba

Pityriasis alba (PA) is a common, benign, localized form of hypopigmentation, which is more common in children than in adults. In children under 12 years of age, prevalence is between 1.9% and 5.25% (2, 48).

The etiology and pathogenesis of PA is not clarified. There is a perception that the disease is more common in dark-skinned people. PA is considered a minor form of atopic dermatitis. Recent studies have indicated the frequent occurrence of PA in individuals who sunbathe without using photoprotection, and the onset of PA as a result of frequent sunbathing. In these patients the low levels of serum copper was detected, and it is known that copper plays an important role in the synthesis of tyrosinase, which may explain the hypopigmentation (6, 48).

The clinical pattern shows unclearly bordered hypopigmented maculae and pityriasis-like desquamation on their surface. The disease begins with an erythematous plaque with elevated edges and desquamation occurs after several weeks. Hypopigmentation can last from 6 months to 7 years. The course is prolonged in patients with atopic dermatitis. PA is an asymptomatic disease, although there may be mild itching. The changes are mainly localized on the head, neck and upper limbs. The most common localizations are the head, the forehead and cheekbones, and it rarely occurs in periorbital and perioral regions, unlike vitiligo. There is a generalized form of atypical PA, which is more often seen in adults, and the changes are localized to the thorax (1, 2, 6, 48).

The therapy involves emollients and corticosteroid creams of low and moderate potency, which have limited effectiveness. The use photoprotective creams is recommended. After a few months or years the condition spontaneously withdraws. In the extensive form in adolescents and adults PUVA therapy is applied (48).

Pityriasis versicolor alba varietas

The cause of the disease is *Pityrosporum ovale* (synonyms: *Pityrosporum orbiculare*, *Malassezia furfur*, *Malassezia ovalis*), part of the normal flora of the skin, which under certain circumstances is transformed from saprophytic yeasts into the mycelal phase and leads to skin diseases. The disease is not contagious and occurs in predisposed peo-

ple, mostly in areas with a humid and warm climate (6, 49).

The clinical picture is presented with café-au-lait or yellowish-brown (*versicolor*) maculae which are circular, oval or irregular in shape, with unclear edges, up to 1 cm in diameter, with pityriasis-like desquamation at the surface (apparent after mild curretage of the macula) and with a tendency to confluence in plaques. The maculae have characteristic distribution sites, with localization on the chest, back and upper half of the upper arm, and in children they occur on the head and neck. In immunodeficient conditions, the skin changes have atypical distribution (inverse) and the face, creases and some parts of the limbs are affected. The maculae become hypopigmented after sunbathing (*Pityriasis versicolor alba varietas*) but hypopigmentation is reversible. An asymptomatic infection is the most common, itching is rarely present. If left untreated, the course becomes chronic and could last for years (49).

Diagnosis is made based on the clinical features and the use of a *Wood*'s lamp, when the yellowish-orange fluorescence of the affected skin is noticed. Native mycological examination with KOH reveals the characteristic appearance of hyphae and spores ("spaghetti and meatballs") (6, 48, 49).

Local therapy is the method of choice, and imidazole compounds are advised (1% Econazole, Ketoconazole 2%) and ciclopiroxolamine in the form of a solution, spray or cream 1-2 times a day for 2-4 weeks. Selenium sulfide 2.5% shampoo and zinc pyrithione shampoo used once a day (foam should be left on the skin 10 minutes), for two weeks. Residual hypopigmentation lasts a few months after healing, which patients should be warned about. The disease may recur, since *Pityrosporum ovale* belongs to the normal saprophytic flora of the skin. Systemic therapy is not recommended because *Pityrosporum ovale* is a normal inhabitant of the skin and cannot be completely removed. For prevention of the disease, a soap is recommended containing Zn and Se sulfide for washing, with mild friction with keratolytics during the summer months as well as the use of a sulfide shampoo with Se and Zn pyrithione, once a week (6, 48, 49).

Piebaldism

Piebaldism is a rare (incidence is 2.5/100000) autosomal dominant disorder caused by the irregular development of melanocytes, which is manifested by the focal absence of melanocytes in the affected skin and hair follicles. The clinical picture is characterized by polyosis and multiple, symmetrical, leukodermic maculae, typically distributed on the sides of the trunk, the anterior abdominal wall and above and below the elbows and knees. Polyosis on the forehead is present in 80-90% of patients. Unlike vitiligo, piebaldism does not appear on the hands, feet and periorificial areas. Hyperpigmented maculae within amelanotic depigmented areas are typical (1, 2, 4).

In patients with piebaldism mutations of C-kit proto-oncogene are found, which encodes the transmembrane receptor and tyrosine kinase activity, causing the omission of migration and differentiation of melanoblasts from the neural tube during embryonic development, in the affected skin areas. Phenotypic characteristics are fully formed at birth and permanent, and usually do not spread afterwards (1, 2). Therapy is symptomatic and includes photoprotection and allows the use of cosmetic camouflage (1, 2).

Tuberous sclerosis

Tuberous sclerosis is an autosomal dominant determined, multisystemic neurocutaneous syndrome, characterized by the formation of multiple hamartomas usually localized in the skin, brain, heart, kidney, liver and lungs. In 2/3 of the patients the disease is the result of sporadic mutations. The char-

acteristic triad consists of deafness, mental retardation and cutaneous angiofibromas, and is seen in only 29% of patients. Early diagnosis is important. At birth or during the first months of life in 97.2% of patients hypopigmented maculae are observed (3 or more), beige-whitish or yellow-whitish in color. They represent a major diagnostic sign. *Ash-leaf spots* or spear-like and polygonal maculae, sized 0.5 to 2 cm, localized mainly on the trunk or upper legs are characteristic. Less commonly confetti macula or macula of dermatome schedule are seen. Size can vary from 4 mm to 12 cm. Later, chagrin plate, facial angiofibromas, periungual fibromas and plaque on the forehead could emerge. Systemic manifestations of tuberous sclerosis fall within the scope of pediatrics (1, 2, 4, 50).

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Case presentation – thyroid lymphoma

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Malignant tumors of the thyroid gland account for about 1% of the newly diagnosed malignant tumors each year, and their incidence in women is twice the incidence in men. According to the WHO classification (2004) thyroid tumors are divided into: carcinoma of the thyroid, adenoma and similar tumors, and other thyroid tumors which include: teratomas, angiosarcomas, paragangliomas and others, as well as primary lymphomas and plasmacytomas. Primary thyroid lymphomas are defined as lymphomas which originate in the thyroid gland. This study presents the case of a 68-year-old patient with a thyroid lymphoma, which caused compression of the airways. In the patient presented there was reduced activity of the thyroid gland. The dominant symptoms were: breathing difficulties, hoarse voice and the enlargement of the thyroid. An ultrasound examination was performed before surgery on the neck, which showed a multinodular thyroid, with compromised and compressed trachea to the right and rear. An emergency surgical procedure was performed to reduce the tumor. Pathohistological diagnosis confirmed diffuse large B cell lymphoma. The aim of the study was to present a patient with a thyroid lymphoma, who had previously not had any immunological changes to the gland, that is, she had not had any chronic lymphocyte thyroiditis, but due to the compressive syndrome it was necessary to perform an emergency surgical procedure to reduce the tumor.

Key words: Thyroid, Lymphoma, Malignant tumor.

Introduction

Malignant tumors of the thyroid gland account for about 1% of the newly diagnosed malignant tumors each year (1) and their incidence in women is twice the incidence in men (2). They most often occur in older patients where the average age of occurrence is 65 years. According to the WHO classification (2004), thyroid tumors are divided into: carcinoma of the thyroid, adenoma and similar tumors, and other thyroid tumors which include: teratomas, angiosarcomas, paragangliomas and others as well as primary lymphomas and plasmacytomas (3). In the group

of carcinomas the most common in the thyroid are papillary (80%), follicular (10%), medullary (5-10%) and the exceptionally rare anaplastic carcinoma (1-2%) (2), whilst the frequency of primary lymphoma is 5% (4). Primary thyroid lymphomas are defined as lymphomas which primarily originate in the thyroid, so this definition excludes lymphomas which affect the thyroid whether by metastasis or by direct spreading from the neighboring lymph nodes. Primary thyroid lymphoma in its late stages may spread to the lymph nodes and other organs, including the gastrointestinal tract, thereby representing a form of mucosa-associated lymphoid tissue (MALT) lymphoma (5).

Primary thyroid lymphomas are usually non-Hodgkin type, whilst primary Hodgkin's disease is very rare (6). Extranodal marginal zone B cell lymphoma (EMZBCL) and diffuse large B cell lymphoma (DLBCL) are lymphomas which most often occur in the thyroid with areas of morphology of a transient form between these two types of lymphoma. Other, especially follicular lymphomas are extremely rate (3). Diffuse large cell B lymphomas are lymphomas which occur most often in the lymph nodes, whilst in 30% of cases they can occur as extra-nodal. The most common extra-nodal site where lymphomas of this type occur is the thyroid (7).

The rapidly growing mass of the thyroid gland in an older woman should always arouse suspicion of a tumor. In the past it was necessary to perform an open biopsy to obtain enough tissue for pathohistological examination (6). However, today diagnosis is made on the basis of cytological analysis of aspirate changes in the thyroid gland, immunocytochemistry, flow cytometry, immunophenotypization of lymphocytes obtained by fine needle aspiration (FNA), and finally pathohistological analysis and molecular genetic analysis (8). If it is a DLBCL lymphoma, that is, a lymphoma with a high degree of malignity, there is usually no problem in establishing a diagnosis from smears obtained by FNA. In cases of unclear diagnosis, if a monomorphic population of small lymphatic cells is found in the smears, it is necessary to repeat the aspiration to obtain sufficient cells for immunophenotypization (6). Differentiating between EMZBCL and lymphocyte thyroiditis, as well as differentiating between other lower level lymphomas than Hashimoto thyroiditis can sometimes be difficult both from cytological smears and histological preparations. Although pathological verification is the gold standard for diagnosis of lymphoma, in rendering a diagnostic decision immunohistochemistry, flow cytometry or genetic molecular analysis are still necessary in addition (3).

Treating large cell lymphomas of the thyroid gland is no different from treatment of any other lymphoma occurring in the lymph nodes (9). Treatment is based on the sub-type of the lymphoma and the scope of the disease. Today, the trend in treatment of large cell lymphomas is for treatment to be selected on the basis of prognostic factors. Treatment of a disease which is limited to the thyroid gland is performed by local regional radiation or surgery. In most patients, treatment is mainly by a standard chemotherapy regime, which consists of Cyclophosphamide, Doxorubicin, Vincristine and Prednisolone (CHOP) in combination with radiation, and must always be under the supervision of an oncologist (11).

The goal of this paper is to present a patient with thyroid lymphoma, who had not had any previous immunological disease of the thyroid gland, that is, chronic lymphocyte thyroiditis, and in whom it was necessary to perform surgery to reduce the tumor due to compression syndrome.

Case presentation

The patient, aged 68, was sent to our institute for examination. A year earlier, the

diagnosis of hypothyroidism had been established in another institution and therapy with 1-thyroxin 100 micrograms once a day prescribed. On clinical examination, a node was palpitated in the left lobe of the thyroid gland, 2 cm in size. The follow up finding of the thyroid-stimulating hormone - TSH was 9.9 mIU/l (0.27-3.75 mlU/l); Free thyroxine - FT4 17 pmol/l (10-20 pmol/l); Thyroglobulin - Tg 0.4 ng/ml (2.0-70 ng/l) and thyroglobulin antibodies - TgAt 9.0 IU/ml (>100 IU/ml). Therapy was recommended with 150 micrograms of l-thyroxin once a day and control of hormonal status in four months.

Three months later the patient was admitted to our Department of Ear, Nose and Throat for breathing difficulties, loss of voice and sudden increase in the size of the thyroid gland. Physical examination established that the thyroid gland was enlarged, with multimodal changes, hard, painless and immobile on swallowing, with bilateral neck lymphadenopathy. Ultrasound showed multimodal changes to the thyroid gland with a compromised and compressed trachea on the right and to the front. On both sides of the neck there were pockets of enlarged lymph nodes. The ultrasound finding indicated a malignant tumor in the thyroid gland. Since compressive syndrome and respiratory insufficiency were present, it was proposed following consultation that tumor reduction and tracheotomy be performed.

The pathohistological diagnosis of the tumorous mass was *Lymphoma malignum diffusum centroblasticum B glandule thyreoideae* – diffuse large cell B lymphoma (figure 1). Immunohistochemical analysis showed that the neoplastic cells were positive for CD20 (figure 2) and bcl-6, whilst they were negative for CD 3, CD 10, CD 23, cyclin D1, TdT and bcl-2.

Post-operative computerized tomography of the neck, thorax and abdomen showed the remains of the tumorous mass, which went from the level of the hyoid bone to the left, paralaryngeally and para- and retropharyngeally, right up to the level of the jugular fossa. It infiltrated the left half of the supraglottic and glottic regions of the larynx, reducing the air space to a latero-lateral diameter of 2 mm. In the distal half of the neck to the left, pathologically enlarged lymph nodes were visible on the jugular chain about 15 and 24 mm in size, which compromised and, it was suspected, also infiltrated the jugular vein internally. In view of the stage of the illness, further therapy was proposed and transfer of the patient to the Oncology, Hematology and Radiotherapy Clinic. During her hospitalization she received a second cycle of

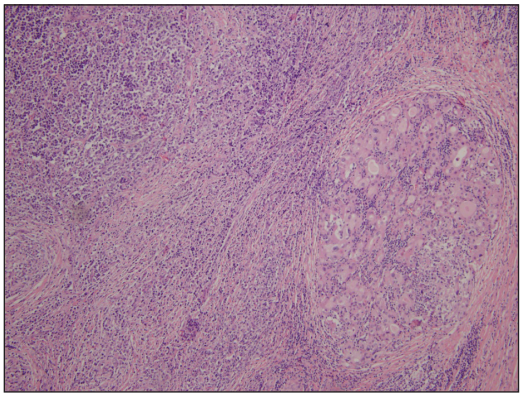

Figure 1 Diffuse large cell B lymphoma of thyroid gland (HE, x 10)

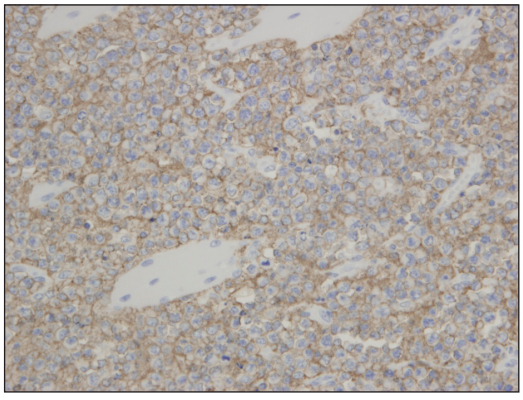

Figure 2 Immunohistochemical analysis - CD20 positive, x 40.

chemotherapy according to the CHOP regime, with the addition of monoclonal CD 20 antibodies. On both occasions she had iatrogenic neutropenia which was treated with granulocyte growth factor and broad spectrum antibiotics. There were also raised levels of the enzymes lactate dehydrogenase, aspartate transaminase and creatinine present in the serum. Despite the therapy applied no regression of the tumorous mass was recorded, so the patient was still unable to swallow spontaneously, but was fed through a nasogastric tube. After two months from admission to hospital in the ENT clinic the illness progressed into the front of the upper mouth and gingiva of the upper jaw, there was loss of body mass and the patient died.

Discussion

Our patient was treated for one year for reduced activity of the thyroid gland, that is, hypothyroidism. The medical history and negative Tg-At finding excluded the existence of Hashimoto thyroiditis. In a relatively short period of time the thyroid gland increased rapidly in size due to the infiltration of neoplastic cells in the form of diffuse nodes, with compression of the airways and resulting respiratory insufficiency. This condition demanded urgent surgical intervention, that is, total thyroidectomy with bilateral dissection of the neck.

The incidence of primary thyroid lymphoma in patients with Hashimoto thyroiditis has increased significantly (7, 10). In fact lymphomas in the thyroid gland in almost all cases occur on the basis of chronic lymphocyte thyroiditis. Graff-Baker et al. believe that there is a pathophysiological connection between autoimmune disorder and thyroid lymphomas. The proposed theory supposes that chronic antigen stimulation is a secondary autoimmune disorder which leads to chronic proliferation of lymph tissue, with resulting mutation leading to the development of lymphoma (9).

Until recently there was concern whether the diagnosis of lymphoma could be established by analysis of a sample obtained by aspiration. Cha et al. (11) showed that this approach is successful in 63% patients with thyroid lymphoma. Aspirates of large cell lymphomas are typically hypercellular with visible individual lymphatic cells, which have a cytological appearance, similar or identical to lymphoma of other sites, and in that case it is simple to reach a diagnosis. In contrast to them, in aspirate smears of the lymphoma marginal zone a mixture is found of small atypical lymphocytes, centrocytes, monocytoid B cells, immunoblasts and plasma cells. As a result of the morphological appearance of the cytological smears, differentiating this type of lymphoma and reactive changes in the lymph node is almost impossible (3). The introduction of molecule technology has led to definite diagnosis and as a result open thyroid biopsy is no longer performed in modern diagnostics (6). Takashima et al. (12) and Daria et al. (13) described the use of chain reaction polymerase in strengthening the immunoglobulin heavy chain to establish the diagnosis of lymphoma. The presence of antigen CD-20 and heavy chain clonality confirm the diagnosis.

Large cell B lymphoma in two patients aged 64 and 50 years, without a previous history of Hashimoto thyroiditis, were described by Akcala et al. (14), saying that due to the sudden increase in size of the thyroid gland and compression of the respiratory path, total thyroidectomy was performed and CHOP chemotherapy applied.

Conclusion

This unusual case of thyroid lymphoma is interesting because it occurred in a patient who did not have Hashimoto thyroiditis.

The surgery was performed because of compression of the airways caused by the rapid increase in size of the thyroid gland due to infiltration by neoplastic cells. After two rounds of chemotherapy there was no regression of the disease and the patient died.

Conflict of interest: The authors declare that they have no conflict of interest. This study was not sponsored by any external organisation.

Authors' contributions: Conception and design: BI and SI; Acquisition, analysis and interpretation of data: BI and AČ; Drafting the article: BI and ŠU, Revising it critically for important intellectual content: AČ and MK.

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Case report

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Peripheral nerve stimulation for treatment of postherpetic neuralgia: A Case report

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Neuromodulation techniques have been successfully used for a variety of neuropathic pain conditions. The aim of this paper is to present a case of the successful use of a subcutaneously placed peripheral nerve stimulator for treatment of intractable postherpetic neuralgia (PHN). A 57-year old man presented with a two-year history of left thoracic pain that developed after a vesicular rash. Focal neuropathic pain had not responded to treatment with multiple analgesic medications and steroid injections. The patient had significant relief following implantation of a peripheral nerve stimulator. This case represents a contribution to the small but growing body of evidence indicating that peripheral nerve stimulation may be an effective option for treatment of PHN not responsive to less invasive modalities.

Key words: Postherpetic neuralgia, Peripheral nerve stimulation, Neuropathic pain.

Introduction

Postherpetic neuralgia is defined as pain persisting or recurring at the site of a herpes zoster rash. The annual incidence has been reported as 11 per 100,000 with a lifetime prevalence of 70 per 100,000 (1). The pain associated with PHN is notoriously difficult to treat (2).

Neuromodulation techniques have been successfully used to treat a variety of neuropathic pain conditions. Electrodes placed in the epidural space have been used to treat thoracic postherpetic neuralgia (3). Subcutaneously placed peripheral nerve stimulation leads have been used to successfully treat a wide range of conditions, including postherpetic trigeminal neuralgia (4). Published cases detailing the use of peripheral nerve stimulation(PNS) in the treatment of postherpetic radicular pain have been sparse (5, 6). We present a case of the successful use of PNS in the treatment of PHN.

Neuromodulation is a reversible blockade or manipulation of pain pathways to modify physiological function and may be applied to the deep brain structures, motor cortex, spinal cord or peripheral nerves (7). The mechanism of pain relief with peripheral nerve stimulation is not clearly understood. One theory is based on the gate control theory of pain, as outlined by Melzac and Wall (8). By this theory, transmission of pain signals to the central nervous system is determined by the balance between large and small fiber activity in the dorsal horn and spinal cord, with small fiber input tending to maintain an open gate to pain transmission and large fiber input tending to close the gate.

Since electrical stimulation depolarizes large fibers before it affects small fibers, the theory suggests that it should be possible for stimulation to halt the transmission of pain signals. This explanation for the function of PNS in pain control is somewhat controversial. An alternative theory is that repetitive stimulation of peripheral nerves may produce excitation failure in c-fiber nociceptors and suppress activity in the dorsal horn of the spinal cord. Stimulation induced blockade of cell membrane depolarization may prevent axonal conduction (9). PNS may also function to decrease the release of excitatory neurotransmitters and increase the release of inhibitory neurotransmitters at the dorsal horn (10).

Spinal cord stimulation has been performed for the treatment of neuropathic pain complaints for 35 years. Though the first studies on implantable PNS therapies were produced in the 1960's, in recent years spinal cord stimulation (SCS) has been the more accepted modality for neuropathic pain (11). Early published reports of PNS showed that a minority of patients had significant pain relief.

Currently most implantable pulse generators are designated as SCS generators and are used for peripheral nerve stimulation on an "off-label" basis. Improvements in electrode and generator technology, as well as the development of percutaneous implantation techniques, have led to a resurgence of interest in the PNS modality.

Recent years have seen PNS used successfully for conditions ranging from postoperative or posttraumatic neuropathies (12) to migraine (13), low back pain (14) and fibromyalgia (15). As part of a larger study of the use of PNS for patients with facial pain, Johnson, et al. (4) reported on 4 patients treated for intractable trigeminal postherpetic neuralgia. Of the four patients, two experienced greater than 50% reduction on the visual analogue scale (VAS) score and were able to reduce their doses of analgesic medication. Harke, et al. (3) studied use of spinal cord stimulation produced via leads placed in the epidural space for treatment of postherpetic neuralgia. In this prospective trial, 23 of 28 patients experienced significant pain relief. Meglio et al. (16) retrospectively studied 10 patients who underwent trials of spinal cord stimulation for postherpetic neuralgia. Of the 10, 6 reported greater than 50% pain relief and underwent implantation. At 15 months follow-up, all 6 patients reported continued pain relief with a mean reported pain relief of 74% (16).

Review of the literature shows only three published cases of the use of PNS in the treatment of PHN related radicular pain (5, 6). Yakovlev and Peterson reported (6) a single case with improvement in pain, functional status, and medication use at six month follow up. Kouroukli et al. (5) reported on two cases with significant improvements in pain and medication use at 6 month and 3 month follow up. Our case represents the fourth reported case of the use of PNS for this indication.

Case report

A 57-year old man with a history of multiple myeloma and chronic renal insufficiency

presented with a two year history of PHN after developing a vesicular rash in the left fifth thoracic dermatome. At the time of initial evaluation, the patient described a burning, lancinating, electrical type of pain that he rated as 9 out of 10 on the visual analogue scale (VAS). The pain was primarily focused in the left periscapular region. The patient reported a history of increased pain when air, clothing, or the shower would touch his skin. Sensory examination revealed allodynia just inferior to the left scapula. No scarring of the skin was evident. Motor function was normal. MRI of the thoracic spine showed no pathology in the region of the pain. He was treated with two series of intercostal nerve blocks, as well as an interlaminar thoracic epidural steroid injection, without relief. He had also been treated with multiple analgesic medications. A lidocaine patch was ineffective, as was a topical compounded cream of amitriptyline, gabapentin, and lidocaine. Gabapentin, pregabalin, and baclofen were tried in succession and were not tolerated due to sedation. Nortriptyline was ineffective. The use of a transcutaneous electric nerve stimulation (TENS) unit only exacerbated his pain. The patient was escalating use of oxycodone and at the time of the electrical stimulation trial was taking 60 to 80 mg daily.

The patient agreed to undergo trial treatment with electrical stimulation therapy. During such a trial, an electrode is placed percutaneously and attached to an external pulse generator. The trial implantation is performed under minimal conscious sedation in order to determine the location of the paresthesia produced and to determine the patient's response to simulation. The patient is then typically discharged with the epidural lead in place with a follow-up visit scheduled several days later. At the time of follow up, the patient's response to stimulation is recorded and the percutaneous lead is removed. Significant pain relief during such a trial indicates that the patient will likely have a good outcome from permanent stimulator implantation. In this case, the trial was carried out with placement of both epidural and subcutaneous leads.

After carefully outlining the area of pain and obtaining informed consent, the patient was taken to the surgical suite. He was placed in a prone position and the lower and mid back was prepped and draped in a sterile fashion. The patient was under light conscious sedation and was monitored throughout the case. After anesthetizing the skin, a 14 gauge Tuohy needle was placed into the epidural space under fluoroscopic guidance at the T12-L1 level using loss of resistance technique. An eight contact electrode (Octrode™) lead (St Jude Medical, Plano, TX) was placed through the Tuohy needle, into the epidural space, with the most superior lead at the midportion of the T1 vertebral body, 0.5 mm to the left of midline. The lead was attached to a St. Jude pulse generator and paresthesia was produced covering the area of left thoracic pain. A second identical lead was placed percutaneously through a 14 gauge Tuohy needle into the subcutaneous space in the area of the left T5 dermatome. The electrode was placed medial to and slightly superior to the location of his most significant pain and allodynia. This electrode was attached to the pulse generator and was tested with the epidural lead inactivated. The subcutaneous lead also produced paresthesia in the distribution of his pain. Tuohy needles and styletes were removed from each site and the percutaneous leads were sutured to the skin. The patient was seen in the recovery room for further fine tuning of programming. The epidural and subcutaneous lead both proved to be equally effective, producing paresthesia and relief of the patient's thoracic pain. The patient was discharged for a 72 hour trial period. He was provided with programs to activate both electrodes individually or both

simultaneously. During the trial period, the patient reported 75% reduction in pain and his opioid requirements only amounted to 25% of his typical daily use. The patient reported equivalent pain relief regardless of which electrode was activated. At the time of follow up, both percutaneous leads were removed.

Six weeks after the trial period, the patient returned for permanent implantation. He had a history of coagulopathy, likely due to his history of multiple myeloma and treatment with chemotherapeutic agents. Due to bleeding during the procedure, the decision was made to proceed with implantation of subcutaneous leads, rather than to perform a laminectomy for epidural placement. The implantation was performed under general anesthesia with assistance from a neurosurgeon. The initial percutaneous lead was placed in a location identical to the subcutaneous trial lead. In order to provide additional coverage of any more lateral symptoms, a second subcutaneous lead was placed approximately 3 inches lateral to the first lead, in the path of the T5 dermatome. A subcutaneous pocket was produced in the upper left buttock and a St Jude Eon Mini rechargeable implantable pulse generator was implanted.

At 6 months follow-up, the patient reported decreased pain and improved quality of life. The maximum pain level had diminished from 10/10 to 4/10 on VAS. The constant burning pain had been completely alleviated but he continued to report occasional lancinating pain. At the time of the last follow-up, he had decreased his use of oxycodone 5 to 10 mg a day.

Discussion

We report a case of the successful use of a subcutaneously placed peripheral nerve stimulator for the treatment of postherpetic neuralgia. This adds to a very small but growing body of literature indicating that peripheral nerve stimulation may be a useful option in the treatment of this relatively common and potentially disabling condition. This study is, of course, limited in that it is a single case report. Prospective studies are needed to establish the utility and safety of PNS for the management of postherpetic neuralgia. The use of PNS entails the risk of nerve injury, bleeding or infection, as well as lead migration or fracture (17). PNS treatment carries a significant up front cost. A cost benefit analysis of SCS and PNS for treatment of chronic pain showed that cost benefits began to accrue after two years of stimulator use (18). There is insufficient data to make a similar claim for the use of PNS alone for chronic pain or for either modality for this particular indication. One clinical limitation of our case was the placement of an additional subcutaneous lead during the permanent implantation. The decision to place this additional lead was made during the surgical procedure when bleeding led to aborting the placement of the epidural lead. Placement of a stimulator lead without trial on a conscious patient may entail additional procedural cost with uncertain clinical efficacy. At this stage in the use of PNS for the treatment of neuropathic pain, guidelines for the correct position or number of subcutaneous leads required or the proper duration of the trial period have not been clearly established.

Conclusion

PNS may be an effective treatment option for patients who have not responded to more conservative treatment. Prospective studies may be useful to determine the long term efficacy of this technology in the treatment of post-herpetic neuralgia, as well as to determine cost effectiveness.

Conflict of interest: The authors declare that they have no conflict of interest. This study was not sponsored by any external organization.

Authors' contributions: Conception and design: SCP; Acquisition, analysis and interpretation of data: SCP; Drafting the article: SCP, AAJ; Revising it critically for important intellectual content: SCP, AAJ.

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The sudden death of a 6.6 year old girl due to anaphylactic shock caused by nonruptured echinococcal cysts of the left ventricle and the liver

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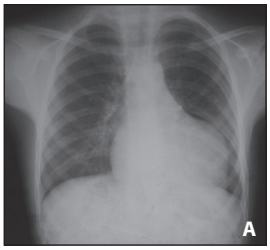
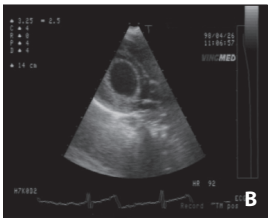
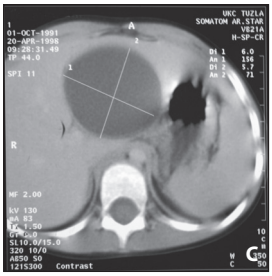
A 6.6-year-old girl presented for left sided cardiac enlargement on chest radiography (Panel A). Three years earlier she had undergone a lobectomy of the lower lobe of the left lung for extraction of an echinococcal cyst. After that she was well, without any medication. There was a 3/6 systolic murmur at the heart apex with ECG signs of left ventricular hypertrophy.

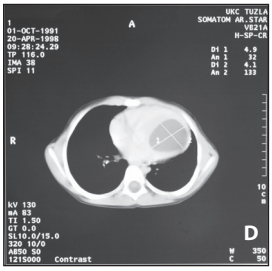
An echocardiogram showed the dilated left ventricle, LVDd 50 mm, filled with a cyst 45 x 35 mm in diameter (Panel B), with moderate mitral regurgitation and normal aortic flow. A CT scan showed a cystic lesion in the lumen of the left ventricle, 50 x 40 mm and in the left lobe of the liver one of 60 x 60 mm (Panels C and D). During the first cycle of treatment with abendazol the child suddenly died due to anaphylactic shock reaction.

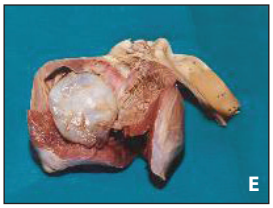
At the autopsy expressed generalized cyanosis was found. The presence was established of an intact echinococcal cyst, 40 x 50 mm in the inventricular septum of the heart with reduction of the lumen and hypertrophy of the wall of the left ventricle, (Panel E). By microscope eosinophilic myocarditis was found. The medial part of the left lobe of the liver was enlarged due to an echinococcal cyst, 100 x 60 mm with the signs of eosinophilic inflammation at the neighbouring parenchyma. On the lungs an oedema was noted, with microscopic bleeding and eosinophilic interstitial inflammation, but with no elements of an echinococcal cyst.











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Apparent Triphasic T-Wave

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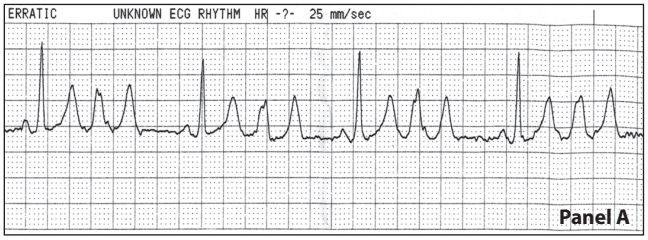
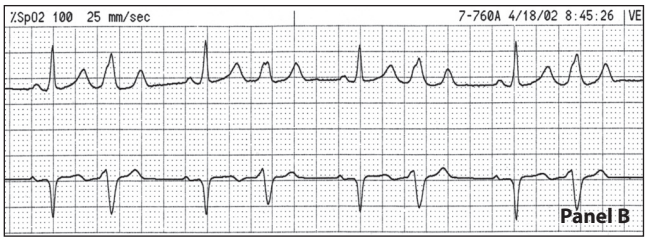
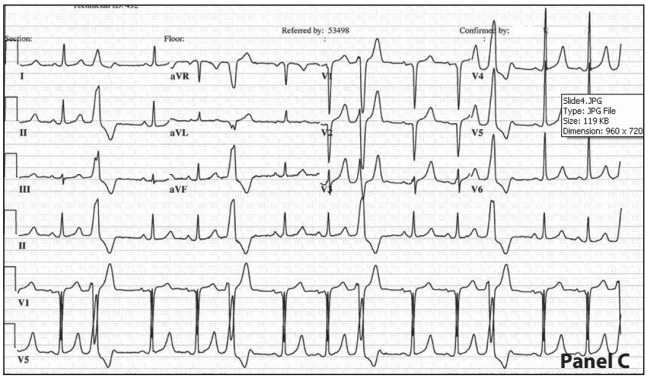
An apparent "triphasic" t-wave was seen in a 59-year-old man upon admission to the intensive care unit. The physician was called to identify the change in rhythm (Panel A). The exam was unremarkable and the patient remained hemodynamically stable. Changing the lead configuration on the monitor revealed bigeminy (Panel B). The wide complex beats most likely represented a triggered ventricular phenomena. However, atrial bigeminy with rate dependent bundle branch could not be ruled out. A 12-lead EKG performed later suggested the diagnosis of ventricular bigeminy (Panel C). This case emphasizes the utility of using multiple leads to differentiate immediately artifacts and distortions commonly seen on rhythm monitors. Performance of 12-lead ECG, a "gold standard" for noninvasive diagnosis of rhythm disturbances, may sometimes be delayed. Changing the leads is a simpler, quicker action and therefore should be performed first. In our case, inspection of other leads was diagnostic and allowed for 12-lead ECG to be performed non-urgently. The unusual appearance of this rhythm may be misleading at first glance. However, interpreting the wide QRS as a second of three t-waves would be very difficult to explain. There are







a number of repolarization abnormalities that can produce notched, bifid, and/or prominent u-waves but a "triphasic" t-wave with this morphology does not fit any known pattern (1). Ventricular bigeminy is commonly associated with hypokalemia, medications such as digoxin and underlying ischemia. The compensatory pause after each VPC tends to perpetuate conditions favoring more bigeminy (2). No underlying electrolyte abnormalities or culprit medications were identified and the arrhythmia resolved spontaneously with no clinical sequela.

Conflict of interest: The authors declare that they have no conflict of interest. This study was not sponsored by any external organisation.

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Adding the PubMed unique identifier to journal article references without increasing the bulkiness of the journal

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Nowadays a large amount of scientific and medical literature can be accessed online in full text format. Although the manuscripts are widely available online, the readers' preference is often for a printed version through personal or library print subscriptions or by printing a copy of the online version for reading convenience.

We propose the idea that adding the PubMed Unique Identifier (PMID) to every PubMed-indexed citation in biomedical journal articles' reference lists will simplify article retrieval by providing a short and unique search string to use with internet search engines. By determining the empty spaces at the end of a set of journal articles and calculating the space required to accommodate the addition of PMIDs for each reference, we tested the hypothesis that adding a PMID to references will not increase the printed size of a journal.

We selected 18 clinical journals in the Mayo Clinic Library, covering most major publishers. One issue of each selected journal was randomly picked from library bookshelves within a range of one year (November-2008 to November-2009). The primary outcome was the increase in number of printed pages for references after adding PMIDs. The space occupied or left blank was measured using a transparent template divided into 20 equal portions, each portion representing 5% of the page.

We analyzed 280 articles from 18 journal issues. The median numbers of articles per issue was 13, ranging from 5 to 38. The number of references per article varied widely from 7 to 199, and the median was 30 references per article. Overall, 92% of references were included in PubMed. The amount of empty space at the end of every article differed between individual journals with an average of 45% (95% CI; 40% to 48%) of empty space.

If the PMID is added to all PubMed references, (generally an eight digit number for current citations) it would increase the length of each article by a median (interquartile range) of 3% (2% to 4%). Journals would still have an average of 40% (95% CI; 35% to 45%) of empty space following each reference list.

Our results suggest that adding a PMID to each cited reference will not substantially increase the length of a printed journal. While the effect of adding the PMID to reference lists in printed publications is minimal, the feature will save readers time in retrieving reference articles and make this process more convenient to users.

Key words: PubMed Unique Identifier, References, Usability.

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Controversies and dilemmas in contemporary psychiatry

Dušan Kecmanović
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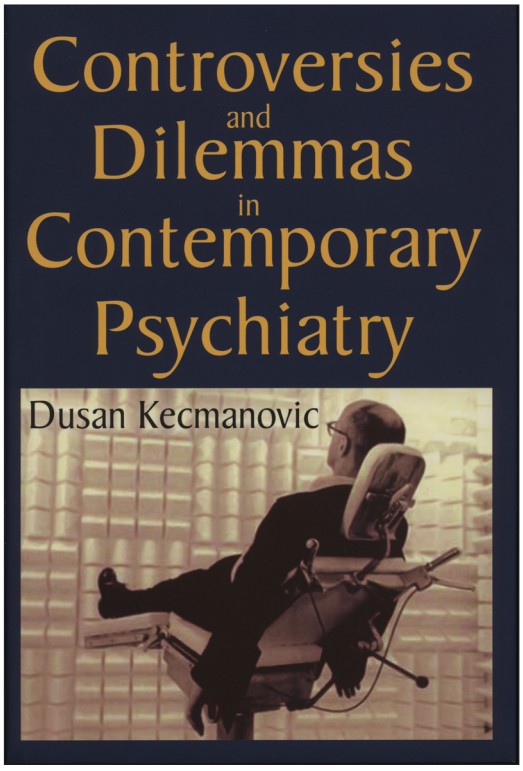

Dusan Kecmanovic is one of the most prominent psychiatrists in the former Yugoslavia. Before leaving Sarajevo in 1993 he was Professor of Psychiatry and Political Psychology at Sarajevo University, and a member of the Academy of Sciences and Arts of Bosnia and Herzegovina. His publications – 24 books and more than 250 papers (as the primary author) published by reputed publishers and in respected journals – made him an internationally acknowledged scholar. As Professor Kecmanovic authored or edited a great many widely read psychiatry textbooks he has contributed enormously to the education of medical students, psychiatric residents, psychiatrists, psychologists and psychiatric social workers in the former Yugoslavia in general and in the Republic of Bosnia-Herzegovina in particular.

Kecmanovic's latest book is entitled *Controversies and Dilemmas in Contemporary Psychiatry*. As the title suggests, it deals with those topics in psychiatry that are debatable in the first place. And there are many of them. As Kecmanovic put it, if we take all the dilemmas out of psychiatry there is not much psychiatry left. Kecmanovic does not take sides. He dissects arguments, casts light on the pros and cons. He explains how some dilemmas might be resolved, and why others are not likely to be resolved because the resolution of them is simply beyond the scope of psychiatry. Kecmanovic is focused on the most important and most intriguing questions that psychiatrists cannot help but confront – no matter whether they are more interested in conceptual issues or in day-to-day clinical practice. Indeed, most psychiatrists are not keen on discussing controversies and dilemmas in contemporary psychiatry because they believe that debating burning psychiatric questions unveils the weaknesses of psychiatry and thereby tarnishes the public image of psychiatry. In this book Kecmanovic forces psychiatrists to face up to the aspects of their job that are only apparently question-free. That is only one of the reasons why the book deserves their attention.

Kecmanovic has chosen to discuss those subject-matters that, in his opinion, contain the main controversies and dilemmas in contemporary psychiatry, such as the definition of mental disorder, mental health, similarities and dif-

ferences between physical diseases and mental disorders, and conceptual discord in psychiatry. When closely inspected, these topics reveal the following controversies and dilemmas*: A mentally ill person lives in two worlds; Psychiatrists do not share the same view of the mind-body relationship; The proponents of each psychiatric model practice psychiatry as though only the model they advocate is legitimate; Psychiatrists are reluctant to acknowledge that they are, inter alia, the guardians of social peace and order; Psychiatrists diagnose mental disorders every day, although there is no generally agreement regarding the definition of mental disorder; Psychiatrists reduce mental health to the absence of mental disorder; Although the official classifications of mental disorders state that there is no difference between somatic disease and mental disorders, the dissimilarities of these pathological phenomena largely outweigh their similarities. Attempts to increase the reliability of mental disorder diagnoses have been made at the cost of the desubjectification and decontectualization of mental disorders; The categorical and dimensional concepts of mental disorders are difficult to reconcile; The methods of causal explanation and meaningful understanding without which psychiatry cannot do, are two essentially different methods; Frequent changes in diagnostic classifications have not been accompanied by a change in the diagnostic work of a large number of psychiatrists throughout the word, and The mental healthcare market reduces the psychiatrist to a psycho-pharmacologist or pharmaco-psychiatrist.*

Kecmanovic devotes due attention to each of these controversies and dilemmas, deliberates on them, and indicates how serious their fall-out is.

I especially enjoyed reading the first chapter entitled: "*Towards a Definition of Mental Disorder*". It is the most comprehensive and most emphatically analytical. Kecmanovic gives his own definition of mental disorder that reads as follows: *A mentally ill person deviates from the prevailing behavior and belief standard in a given environment; one or more mental functions are impaired in such persons, and this is preceded or followed by psychological dysfunction; their deviation and the impairment of one or more mental functions happen against their will, and causes the mental suffering of the respective person*. The second chapter (*From Normality to Mental Health)* deals with mental health. According to the author, there are three key concepts of mental health: the clinical-pragmatical, the positive psychology view of mental health, and the humanistic-philosophical approach to mental health. Kecmanovic favors the humanistic-philosophical approach. He claims that only this approach is in tune with the very nature of human beings. Unlike some renowned scholars, he contends that physical diseases and mental disorders differ in many regards. His position on this topic is expounded in the third chapter (*Physical Diseases and Mental Disorders: Should They Be Differentiated?*). Kecmanovic has managed to trace a large number of features of physical diseases and mental disorders that do not allow us to put them on the same footing, not to mention to consider them as one and the same. In the last chapter the author analyses the origin and consequences of the legitimate existence of several general conceptions or models in psychiatry. Psychiatric models are incommensurable to the point of having nearly nothing in common, which virtually means that it is hard even to imagine a dialogue between them. Given the deleterious effects that conceptual discord has on psychiatric practice it is astonishing how little attention psychiatrists pay to it. Actually they take it for granted. One thing is for sure, psychiatrists are not competent to resolve the problem of conceptual discordance in psychiatry as its resolution depends on the answer to the mind-body puzzle. And this question is a pre-eminently philosophical question.

In conclusion, the book *Controversies and Dilemmas in Contemporary Psychiatry* is a must-read. It provides a balanced stance between psychiatry iconoclasts and psychiatry iconoclasters (or iconophiles), that is, between psychiatry critics and psychiatry defenders. There is no better way to describe Kecmanovic's general position, as far as psychiatric controversies are concerned, than to quote him. He writes: "To designate and analyze the quandaries in psychiatry does not mean to put psychiatry in question. It should rather be the mark of a reflective view of psychiatry".

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Half a century of "Pula International Neuropsychiatric Congresses"

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The regular yearly professional and scientific meetings, the Pula Symposia (now Congresses), for more than 50 years, demonstrates their special lasting value and deserves great respect. Founded during the "Cold War" (in 1961) on the "crossroads of worlds and ideologies," the International Neuropsychiatric Pula Congresses (INPC) are also a good example of quality in cooperation between psychiatrists and neurologists in Central and South-East Europe: so they hold a special position amongst other similar scientific and professional manifestations. Boško BARAC and Helmut Lechner (2006) found the best explanation for the position of the INPC: *"They have promoted interdisciplinary collaboration between what are nowadays independent disciplines: neurology and psychiatry, with the borderline and collaborating medical and non-medical disciplines, reflecting advances in medical and neurological sciences and following the professional and technical developments in medicine"*. Congresses in Pula have been a great opportunity for all participants to exchange their experiences in all aspects of work with neurological and mental patients. Speakers, prominent and experienced lecturers from Europe and distant countries (Germany, Austria, Italy, Switzerland), but also from Belgium, UK, USA, Australia, Czech Republic, Slovakia, Hungary, Scandinavia, Israel and Arab countries, Bulgaria, from all Republics of the former Yugoslavia and many other countries, have given a wide spectrum of current valuable scientific and practical information, to be implemented in the participating countries. However, the exchange of experience and the implementation of new diagnostic and therapeutic methods are not the only achievements of the Pula congresses: they are also a place for new communication established among professionals from various parts of the world. They provide the chance for communication of young neuro-psychiatrists with their colleagues from the developed European centers. Through practical work on different research projects and problems, they have become more competent and skilled scientists and practical doctors, implementing new knowledge in their home institutions.

After 51 years the INPC represent, without any doubt, a vital professional and educational meeting place for neurologists and psychiatrists seriously involved in the field. The reputation of lecturers, selection of actual topics, fruitful and constructive discussions are the aspects why the INPC brought attention of young and perspective neurologists and psychiatrists in this part of Europe and of the world.

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3. Loga S. Reflections of the International Neuropsychiatric Pula Congresses (INPC) to neurology and psychiatry in Bosnia and Herzegovina. In: 50 years International Neuropsychiatric Pula Congresses. Memorial book 1961-2010. Udruga za neuropsihijatriju, Zagreb; 2010. pp. 252-257.

Peer Reviewers for Acta Medica Academica, Vol. 40.

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International publications of authors from Bosnia and Herzegovina in Current Contents indexed publications in the first half of 2011*

Bogunić F, Šiljak-Yakovlev S, Muratović E, Ballian D. Different karyotype patterns among allopatric Pinus nigra (Pinaceae) populations revealed by molecular cytogenetics. Plant Biol (Stuttg). 2011 Jan;13(1):194-200. doi: 10.1111/j.1438-8677.2010.00326.x.

Faculty of Forestry, University of Sarajevo, Sarajevo, Bosnia and Herzegovina.

To examine variation and taxonomic recognition of Pinus nigra (European black pine) at the intraspecific level, chromosomal distribution of 5S and 18S-5.8S-26S rDNA loci revealed by fluorescent in situ hybridisation (FISH) and fluorochrome banding with chromomycin A(3) and DAPI were analysed among allopatric populations belonging to different subspecies. Despite prevalent opinion on predominantly conserved and homogenous conifer karyotypes, several patterns were observed. Surprisingly, interstitial 18S rDNA loci and DAPI heterochromatin staining after FISH showed variations in distribution and localisation. Three subspecies shared a pattern with nine 18S rDNA loci (ssp. nigra, pallasiana and laricio) while ssp. dalmatica and salzmannii had eight rDNA loci. DAPI banding displayed two patterns, one with a high number of signals (ssp. nigra, pallasiana and dalmatica) and the other with a lower number of signals (ssp. salzmannii and laricio). We conclude that our results cannot provide proof for either classification scheme for the P. nigra complex, but rather demonstrate the variability of different heterochromatin fractions at the intraspecific level.

Delibegović S, Iljazović E, Katica M, Koluh A. Tissue reaction to absorbable endoloop, nonabsorbable titanium staples, and polymer Hem-o-lok clip after laparoscopic appendectomy. JSLS. 2011 Jan-Mar;15(1):70-6.

Department of Surgery, University Clinic Center Tuzla, Tuzla, Bosnia and Herzegovina.

BACKGROUND AND OBJECTIVES: The standard technique for securing the base of the appendix during laparoscopic appendectomy is by absorbable endoloop ligature, although clinical reports favor the use of the stapler. Nonabsorbable Hem-o-lok clips have been shown to be an alternative technique to this. However, it is currently not clear whether nonabsorbable clips have any effects on the intestine or promote infection in the surgical area. MATERIALS AND METHODS: Sixty Wistar albino rats were randomized into 3 treatment groups: group I (n=20) the base of the appendix was secured by endoloop 2-0 ligature; group II (n=20) dissection of the appendix was performed by a 45-mm thick stapler; and group III (n=20) the base of the appendix was secured by a Hem-o-lok plastic clip. The animals were sacrificed on the 14th and 28th days after surgery. The secured stump was used for histopathological examination. RESULTS: There were no significant differences in histopathological changes observed on the 14th postoperative day between the groups. On the 28th postoperative day, it was proved that mild and moderate inflammation is more frequent in the endoloop and Hem-o-lok groups than in the stapler group. Reaction to a foreign

*Data for this survey were collected from PubMed database using the keywords Bosnia and Herzegovina and 2011.

body is more frequent in the endoloop than in stapler and Hem-o-lok groups. CONCLUSION: The mildest postoperative inflammatory changes were seen in the stapler group, followed by the Hem-o-lok group. However, because of the price of the plastic clip and the simplicity of its application, its use is still favored during laparoscopic appendectomy.

Dilić M, Nalbantić AD, Arslanagić A, Huskić J, Brđanović S, Kulić M, Hodžić E, Sokolović Š, Zvizdić F, Džubur A. Biphasic and monophasic pattern of brain natriuretic peptide release in acute myocardial infarction. Coll Antropol. 2011 Mar;35(1):155-9.

Sarajevo University Clinical Centre, Institute for Vascular Disease, Sarajevo, Bosnia and Herzegovina.

This study evaluated brain natriuretic peptide (BNP) release in acute myocardial infarction (AMI), absolute values as well as pattern of its release. There are two different patterns of BNP release in AMI; monophasic pattern--concentration in the first measurement is higher than in the second one, and biphasic pattern--concentration in the first measurement is lower than in the second one. We observed significance of biphasic and monophasic pattern of BNP release related to diagnostic and prognostic value. We included in this prospective observational study total of 75 AMI patients, 52 males and 23 females, average age of 62.3 +/- 10.9 years with range of 42 to 79 years. BNP was measured and pattern of its release was evaluated. In AMI group BNP levels were significantly higher than in controls (462.88 pg/mL vs. 35.36 pg/mL, $p < 0.001$). We found statistically significant real negative correlation ($p < 0.05$) between BNP concentration and left ventricle ejection fraction (LVEF) with high correlation coefficient ($r = -0.684$). BNP concentrations were significantly higher among patients in Killip class II and III compared to Killip class I; Killip class I BNP = 226.18 pg/mL vs. Killip class II 622.51 pg/mL vs. Killip class III 1530.28 pg/mL, $p < 0.001$. BNP concentrations were significantly higher in patients with; (i) myocardial infarction vs. controls; (BNP 835.80 pg/mL vs. 243.03 pg/mL); (ii) in pts with positive major adverse cardiac events (MACE) vs. negative MACE (BNP 779.08 pg/mL vs. 242.28 pg/mL, $p < 0.001$); (iii) in pts with positive compared to negative left ventricle (LV) remodelling (BNP 840.77 pg/mL vs. 341.41 pg/mL, $p < 0.001$). Group with biphasic pattern of BNP release had significantly higher BNP concentration compared to monophasic pattern group. In biphasic pattern group we found significant presence of lower LVEF, Killip class II and III, LV remodelling and MACE. We found that BNP is strong marker of adverse cardiac events in patients presenting with a myocardial infarction. In our AMI group we found significant elevation of BNP and it is suspected that second peak secretion is not only due to systolic dysfunction and subsequent remodeling of LV but also due to impact of ischaemia. Patients with biphasic pattern probably have worse prognosis due to severe coronary heart disease. Besides its diagnostic role as a simple blood marker of systolic function, BNP is also important prognostic marker who helps making clinical decision about early invasive vs. conservative management.

Fatušić Z, Hudić I, Sinanović O, Kapidžić M, Hotić N, Musić A. Short-term postnatal quality of life in women with previous Misgav Ladach caesarean section compared to Pfannenstiel-Dorffler caesarean section method. J Matern Fetal Neonatal Med. 2011 Sep;24(9):1138-42. Epub 2011 Jan 13.

Clinic for Gynecology and Obstetrics, University Clinical Center Tuzla, Tuzla, Bosnia and Herzegovina.

Objective. To examine whether short-term postnatal health-related quality of life differed among women after different methods of cesarean sections. Methods. One hundred forty-five women were evaluated with previous CS (85 by Misgav Ladach and 60 by Pfannenstiel-Dörffler). Short-time quality of life was measured using the Croatian version of Short Form Health Survey (SF - 36). Short-term postoperative recovery was assessed using two criteria: febrile morbidity and degree of pain. Incidence of peritoneal adhesions was assigned using Bristow scoring system. Results. Four weeks after delivery women with previous Misgav Ladach cesarean section significantly scored higher on the bodily pain (72.4 vs. 56.7, $p<0.05$), social functioning (71.5 vs. 60.4, $p<0.05$), and the vitality (61.7 vs. 50.3, $p<0.05$) subscales. These differences disappeared in the second assessment (12-weeks postpartum) except in the bodily pain (74.7 vs. 61.2, $p<0.05$) subscale. There was a significant trend toward a higher requirement for postoperative analgesics in the Pfannenstiel-Dörfler group (doses: 5.4 vs. 8.7, $p<0.05$; hours: 17.9 vs. 23.3, $p<0.05$), and they had a significantly higher rate of febrile morbidity than the Misgav Ladach group (5.7 vs. 9.4%, $p<0.05$). Hospitalization time was reduced in the Misgav Ladach group (4.2 vs. 7.3, $p<0.05$). The incidence of adhesions was significantly lower in patients who had undergone a previous operation using the original Misgav Ladach method (0.47 vs. 0.77, $p<0.05$). Conclusion. Misgav Ladach cesarean section method might lead to better short-time quality of life resulting in reducing postoperative complications compared to Pfannenstiel-Dörfler cesarean section method.

Galić G, Tomić M, Galešić K, Kvesić A, Šoljić M,

Lončar Z, Valenčić M, Martinović Ž, Vučkov Š. The etiological relation between serum iron level and infection incidence in hemodialysis uremic patients. Coll Antropol. 2011 Mar;35(1):93-101.

University of Mostar, Mostar University Clinical Hospital, Mostar, Bosnia and Herzegovina.

Through the treatment of anaemia in dialysis patients part of the iron ions remain free in the serum which is at the bacterias disposal for growth and the strengthening of their virulence. The linear relation of the increased serum iron level and tissue iron stores in the body and the infection incidence in dialysed patients has become more emphasised. The need of a clearly defined upper threshold of the serum iron concentration limit has been mentioned in scientific journals intensely, and consequently the demand for more precise professional instructions for anaemia treatment. For the purpose of participating in these professional and scientific discussions, we have observed the relation between the iron overload of the organism and complication incidence in 120 of our haemodialysis uremic patients, with special emphasis on infections. It has been established that the sepses incidence is much higher in patients with a serum ferritin concentration above 500 microg/L, than in those patients with a ferritin level lower than the mentioned value (2 = 7.857, p = 0.005). The incidence of vascular access infection is significantly higher in those patients with a serum ferritin level above 500 microg/L than in those patients with a ferritin level lower than the mentioned value (Chi2 = 23.186, p = 0.001). Furthermore, it has been determined that the incidence of total infection in patients is 3.8 episodes per 100 patients months, which is in accordance to the referral values of other authors. CONCLUSION--In the analysis of the achieved results, it has been determined that the infection incidence is significantly higher in dialysed patients with a serum iron level higher than 500 g/L, than in those patients with lower values.

Hodžić S, Hukić M, Franciosa G, Aureli P. The pathogenic potential of different pulsed-field gel electrophoresis types of Listeria monocytogenes strains isolated from food in Northeast Bosnia and Herzegovina. Vector Borne Zoonotic Dis. 2011 Sep;11(9):1279-83. Epub 2011 May 25.

Faculty of Science, University of Tuzla, Tuzla, Bosnia and Herzegovina.

Listeria monocytogenes is often present in meat and meat products that are sold in the area of northeast Bosnia and Herzegovina. The major objective of this study was to examine the virulence of L. monocytogenes strains isolated from these types of food in that geographic area. Polymerase chain reaction was used to detect eight genes responsible for virulence of this pathogen, namely, prfA, inlA, inlB, hly, plcA, plcB, actA, and mpl. All examined isolates were confirmed to possess the eight virulence genes. Ten different pulsed-field gel electrophoresis (PFGE) macrorestriction profiles were recognized among 19 L. monocytogenes strains after restriction with two different endonucleases (ApaI and AscI). The pathogenicity of three different PFGE types of L. monocytogenes was confirmed through in vivo tests, which were performed on female white mice (Pasteur strain), and it ranged from 3.55 × 10(8) LD50 to 1.58 × 10(10) LD50. All of the three different PFGE types of L. monocytogenes were regarded as moderately virulent in relation to the reference strain L. monocytogenes Scott A. This result might be one of the reasons for the absence of reported listeriosis in northeast Bosnia and Herzegovina, despite the high degree of food contamination with this pathogen.

Hudić I, Radončić F, Fatušić Z. Incidence and causes of maternal death during 20-year period (1986-2005) in Tuzla Canton, Bosnia and Herzegovina. J Matern Fetal Neonatal Med. 2011 Oct;24(10):1286-8. Epub 2011 Feb 28.

Clinic for Gynecology and Obstetrics, University Clinical Center Tuzla, Tuzla, Bosnia and Herzegovina.

The purpose of this study was to determine how increased inaccessibility of health care during the war reflected on maternal outcomes in Tuzla Canton, Bosnia and Herzegovina. We retrospectively collected data from the databases of University Department for Gynecology and Obstetrics and Department of Patology. During war years (1992-1995), the rate of maternal mortality was 87/100,000 births, in the prewar (1986-1991) was 49/100,000 births, in the postwar (1996-2000) was 50/100,000 births, and in the 2001-2005 period was 23/100,000 births. Maternal mortality was significantly higher during the war, mainly due to lower adequacy and accessibility of health care, explosive injuries, and inadequate nutrition.

Hukić M, Hübschen JM, Šeremet M, Salimović-Bešić I, Mulaomerović M, Mehinović N, Karakaš S, Charpentier E, Muller CP. An outbreak of rubella in the Federation of Bosnia and Herzegovina between December 2009 and May 2010 indicates failure to vaccinate during wartime (1992-1995). Epidemiol Infect. 2011 Apr 28:1-7. [Epub ahead of print]

Institute of Clinical Microbiology, Clinical Centre University of Sarajevo, Sarajevo, Bosnia and Herzegovina.

SUMMARYA rubella outbreak involving 1900 cases was recorded in the Federation of Bosnia and Herzegovina between mid-December 2009 and the end

of May 2010. Sera from 389 suspected rubella cases were examined for the presence of rubella-specific IgM and IgG antibodies. A total of 32 throat swabs from suspected rubella cases were tested by RT-PCR and were used to attempt virus isolation. Most patients (945/1900, 49·73%) had never received rubella vaccination or had an unknown vaccination status (563/1900, 29·63%). About 45% (178/389) of suspected rubella patients were IgM positive. From 13 of the throat swabs a virus isolate and E1 gene sequences attributed to genotype 2B were obtained. The rubella outbreak was due to failure to vaccinate during the war period (1992-1995) and emphasizes the need for additional vaccination opportunities.

Imamović G, Zerem E, Osmanović E. Survival of living-related kidney graft recipients in the era of modern immunosuppressive treatment. Ann Saudi Med. 2011 May-Jun;31(3):279-83.

Department of Nephrology and Dialysis, University Clinical Center Tuzla, Tuzla, Bosnia and Herzegovina, Bosnia.

BACKGROUND AND OBJECTIVES: Currently, there is no consensus about immunosuppressive therapy following kidney transplantation. Acute rejection rates and allograft survival rates are the clinical outcomes traditionally used to compare the efficacy of various immunosuppressive regimens. Therefore, we conducted this study to evaluate whether patient survival rates improved in the era of modern immunosuppressive treatment during living-related kidney transplantation. DESIGN AND SETTING: Retrospective cohort study in a university-based tertiary internal medicine teaching hospital performed between 1999 and 2009 and patients followed up to 7 years. PATIENTS AND METHODS: Survival rates were assessed in 38 patients receiving basiliximab and mycophenolate mofetil (regimen A) and 32 patients receiving antithymocyte globulin and azathioprine (regimen B). The rest of the regimen (cyclosporine A and steroids) remained the same. A secondary end point was acute rejection episode. RESULTS: Seven-year survival rates were 100% and 72% (P=.001) and 7-year acute rejection-free survival rates were 82% and 53% (P=.03), in groups A and B, respectively. CONCLUSION: Long-term survival after living-related kidney transplantation has improved in the era of modern immunosuppressive treatment.

Klupka-Sarić I, Peterlin B, Lovrečić L, Sinanović O, Vidović M, Sehanović A, Čizmarević NS, Sepčić J, Kapović M, Ristić S. Angiotensin-Converting Enzyme Gene Polymorphism in Patients with Multiple Sclerosis from Bosnia and Herzegovina. Genet Test Mol Biomarkers. 2011 Jun 23. [Epub ahead of print]

Department of Neurology, School of Medicine, University of Mostar, Mostar, Bosnia and Herzegovina.

Background: Increased activity of angiotensin-converting enzyme (ACE) in the blood and cerebrospinal fluid of patients with multiple sclerosis (MS), and the inhibition of ACE in experimental autoimmune encephalomyelitis, suggested that ACE may play a role in the pathogenesis and progression of MS. We recently published the first report on the potential association of MS and ACE I/D polymorphism in Slovenian and Croatian patients with MS, in which it was shown that the DD genotype might contribute to a higher risk of developing MS in men. To confirm these findings in a similar ethnic population, we analyzed ACE I/D gene polymorphism in patients with MS from Bosnia and Herzegovina. Subjects and Methods: One hundred and seventy patients with MS and 170 healthy controls were genotyped by the polymerase chain reaction method. Results: There was no significant difference in the distribution of ACE I/D genotypes (p=0.783) or in the allelic frequencies (p=0.538) between patients with MS and control subjects. When patients with MS were stratified by sex, no statistically significant differences in allele or genotype distributions were observed. Finally, there was no indication of an impact of the ACE I/D genotype on disease course or severity. Conclusion: The ACE I/D polymorphism is not a risk factor for development of MS, nor does it contribute to disease severity in this Bosnia and Herzegovina population.

Lukić-Bilela L, Perović-Ottstadt S, Walenta S, Natalio F, Plese B, Link T, Müller WE. ATP distribution and localization of mitochondria in Suberites domuncula (Olivi 1792) tissue. J Exp Biol. 2011 May 15;214(Pt 10):1748-53.

Laboratory for Molecular Genetics of Natural Resources, Institute for Genetic Engineering and Biotechnology, Gajev Trg 4, Sarajevo, Bosnia and Herzegovina.

The metabolic energy state of sponge tissue in vivo is largely unknown. Quantitative bioluminescence-based imaging was used to analyze the ATP distribution of Suberites domuncula (Olivi 1792) tissue, in relation to differences between the cortex and the medulla. This method provides a quantitative picture of the ATP distribution closely reflecting the in vivo situation. The obtained data suggest that the highest ATP content occurs around channels in the sponge medulla. HPLC reverse-phase C-18, used for measurement of ATP content, established a value of 1.62 μmol ATP g^{-1} dry mass in sponge medulla, as opposed to 0.04

μmol ATP g^{-1} dry mass in the cortex, thus indicating a specific and defined energy distribution. These results correlate with the mitochondria localization, determined using primary antibodies against cytochrome oxidase c subunit 1 (COX1) (immunostaining), as well as with the distribution of arginine kinase (AK), essential for cellular energy metabolism (in situ hybridization with AK from S. domuncula; SDAK), in sponge sections. The highest energy consumption seemed to occur in choanocytes, the cells that drive the water through the channel system of the sponge body. Taken together, these results showed that the majority of energetic metabolism in S. domuncula occurs in the medulla, in the proximity of aqueous channels.

Marjanović D, Konjhodžić R, Butorac SS, Drobnič K, Merkaš S, Lauc G, Primorac D, Anđelinović Š, Milosavljević M, Karan Ž, Vidović S, Stojković O, Panić B, Vučetić Dragović A, Kovačević S, Jakovski Z, Asplen C, Primorac D. Forensic DNA databases in Western Balkan region: retrospectives, perspectives, and initiatives. Croat Med J. 2011 Jun;52(3):235-44.

Institute for Genetic Engineering and Biotechnology, Sarajevo, Bosnia and Herzegovina.

The European Network of Forensic Science Institutes (ENFSI) recommended the establishment of forensic DNA databases and specific implementation and management legislations for all EU/ENFSI members. Therefore, forensic institutions from Bosnia and Herzegovina, Serbia, Montenegro, and Macedonia launched a wide set of activities to support these recommendations. To assess the current state, a regional expert team completed detailed screening and investigation of the existing forensic DNA data repositories and associated legislation in these countries. The scope also included relevant concurrent projects and a wide spectrum of different activities in relation to forensics DNA use. The state of forensic DNA analysis was also determined in the neighboring Slovenia and Croatia, which already have functional national DNA databases. There is a need for a 'regional supplement' to the current documentation and standards pertaining to forensic application of DNA databases, which should include regional-specific preliminary aims and recommendations.

Pašić A, Tahirović H, Hadžibeganović M. Incidence of asthma in children in Tuzla Canton--Bosnia and Herzegovina. Coll Antropol. 2011 Jun;35(2):299-303.

Tuzla University Clinical Center, Department of Pediatrics, Tuzla, Bosnia and Herzegovina.

Asthma is one of the most common chronic diseases whose incidence shows constant growth in childhood. The objective of this work was to look into asthma incidence in children in relation to their age group and sex in a retrospective study, at Tuzla Canton area. The study comprised children of both sexes, age 0-14 who fell sick with asthma within the period from January 1st 2003 to December 31st 2007. The overall incidence and the incidence in relation to age group and sex was calculated as the number of children suffering from asthma, within the age group 0-14 years per 1000 children of the same age group in the Tuzla Canton. Asthma was diagnosed in 277 children (66.1% male and 33.9% female). The difference between asthma frequency in boys and girls was significant (chi2 = 56.16; df = 1; $p < 0.0001$). The average difference in proportion between the boys and girls was 32.2% (95% CI = 24.32-40.08). From this sample group the boys had a 3.8 times greater risk (OR = 3.79; %95 CI = 2.67-5.39) of contracting asthma. The average rate of incidence of asthma for both sexes in the observed period was 0.67/1000 (95% CI; 0.6-0.7; for boys 0.86/1000; for girls 0.47/1000). There was a statistically significantly higher incidence of asthma in boys in relation to girls ($t = 6.3836$, df = 32; $p < 0.0001$). The epidemiological data obtained could be useful for early detection and adequate treatment of children with asthma in the mentioned area.

Prohić A, Kasumagić-Halilović E. Identification of Malassezia species from immunocompetent and immunocompromised patients with seborrheic dermatitis. Eur Rev Med Pharmacol Sci. 2010 Dec;14(12):1019-23.

Department of Dermatovenerology, University Clinical Center of Sarajevo, Sarajevo, Bosnia and Herzegovina.

BACKGROUND AND OBJECTIVES: Differences in prevalence, clinical and histological manifestations between seborrheic dermatitis (SD) in immunocompetent and immunocompromised patients suggest that these two populations might also differ in a spectrum of isolated Malassezia species. The purpose of our study was to analyse the prevalence of Malassezia species in immunocompromised and non-immunocompromised patients with SD and to examine if the range of isolated yeasts varies between these two study groups. PATIENTS AND METHODS: Specimens were taken from 50 patients with SD: 30 without any underlying disease and 20 with confirmed immunosuppression. The samples were obtained by scraping the skin surface of the scalp and trunk lesions of all subjects and then incubated on modified Dixon agar. The yeasts isolated were identified by their morphological and physiological properties according to Guillot et al method. RESULTS: In both groups, the most commonly isolated species from the scalp

lesions were Malassenzia restricta and Malassenzia globosa, the later being the most common species isolated from lesional trunk skin. No significant differences were found between immunocompromised and immunocompetent patients from both sampled sites. CONCLUSIONS: There is no difference in the distribution of Malassezia species isolated from SD lesions between immunocompetent and immunocompromised patients. However, the much higher percentage of positive cultures in immunocompromised patients confirms that impaired cellular immunity may facilitate fungal survival on the skin.

Redžić A, Smajilagić A, Aljičević M, Berberović Lj. In vivo osteoinductive effect and in vitro isolation and cultivation bone marrow mesenchymal stem cells. Coll Antropol. 2010 Dec;34(4):1405-9.

Institute for Biology and Human Genetics, Medicine Faculty, University of Sarajevo, Sarajevo, Bosnia and Herzegovina.

Bone marrow contains cell type termed mesenchymal stem cells (MSC), first recognized in bone marrow by a German pathologist, Julius Cohnheim in 1867. That MSCs have potential to differentiate in vitro in to the various cells lines as osteoblast, chondroblast, myoblast and adipoblast cells lines. Aims of our study were to show in vivo capacity of bone marrow MSC to produce bone in surgically created non critical size mandible defects New Zeland Rabbits, and then in second part of study to isolate in vitro MSC from bone marrow, as potential cell transplantation model in bone regeneration. In vivo study showed new bone detected on 3D CT reconstruction day 30, on all 3 animals non critical size defects, treated with bone marrow MSC exposed to the human Bone Morphogenetic Protein 7 (rhBMP-7). Average values of bone mineral density (BMD), was 530 mg/cm3, on MSC treated animals, and 553 mg/cm3 on control group of 3 animals where non critical size defects were treated with iliac crest autologue bone graft. Activity of the Alkaline Phosphatase enzyme were measurement on 0.5, 14, 21, 30 day and increased activity were detected day 14 on animals treated with bone marrow MSCs compared with day 30 on iliac crest treated animals. That results indicates strong osteoinduction activity of the experimental bone marrow MSCs models exposed to the rhBMP-7 factor Comparing ALP activity, that model showed superiorly results than control group. That result initiates us in opinion that MSCs alone should be alternative for the autolologue bone transplantation and in vitro study we isolated singles MSCs from the bone marrow of rat's tibia and femora and cultivated according to the method of Maniatopoulos et all. The small initial colonies of fibroblast like cells were photo-documented after 2 days of primary culture. Such isolated and cultivated MSCs in future studies will be exposed to the growth factors to differentiate in osteoblast and indicate their clinically potential as alternative for conventional medicine and autologue bone transplantation. That new horizons have potential to minimize surgery and patient donor morbidity, with more success treatment in bone regenerative and metabolism diseases.

Rifatbegović M, Maksimović Z, Hulaj B. Mycoplasma ovipneumoniae associated with severe respiratory disease in goats. Vet Rec. 2011 May 28;168(21):565. Epub 2011 May 24.

Department of Microbiology and Immunology, Veterinary Faculty, University of Sarajevo, Bosnia and Herzegovina.

No abstract available.

Selimović E, Ibrahimagić-Šeper L, Petričević N, Nola-Fuchs P. Pain relieve after impacted wisdom teeth extraction dependent on the drug therapy. Coll Antropol. 2011 Mar;35(1):133-6.

Public Institute Health Center Zenica, Zenica, Bosnia and Herzegovina.

Purpose of this study was to compare the effects of combined therapy using nonsteroid anti-inflammatory analgetics and corticosteroids, and the effects of the mono-therapy with same drugs for post-operative pain after surgical removal of the impacted mandibular third molar. The study was completed at the Department of Oral Surgery and at the Department of Dental Medicine of the Public Institute Health Center Zenica in Zenica. The research included 60 patients divided into 3 groups using random selection, including both sexes. Age range was between 18 and 45 years. All participants came without any pain or other inflammatory symptoms at the time of oral surgical intervention. Two medicaments were prescribed after the impacted tooth removal: 15 mg of nonsteroid anti-inflammatory analgesic drug (Meloxicam, Bosnalijek, BiH) and 32 mg Methylprednisolone (corticosteroid, Bosnalijek, BiH). Both medicaments were applied per os, according to schedule determined by the research protocol. The level of post-surgical pain was evaluated by the 1-10 visual analog scale (VAS). One way ANOVA was made with Tuckey post-hoc tests. Statistically significant difference ($p < 0.05$) was found between the group treated with mono therapy and the group treated with combined therapy. Application of monotherapy using only corticosteroids or only nonsteroid anti-inflammatory pain-killers was less effective compared to the combined therapy with both medicaments after surgical removal of the impacted

mandibular third molar.

Semiz S, Dujić T, Ostanek B, Velija-Ašimi Z, Prnjavorac B, Bego T, Malenica M, Mlinar B, Heljić B, Marc J, Čaušević A. Association of NAT2 polymorphisms with type 2 diabetes in a population from Bosnia and Herzegovina. Arch Med Res. 2011 May;42(4):311-7.

Department of Biochemistry and Clinical Analysis, Faculty of Pharmacy, University of Sarajevo, Bosnia and Herzegovina.

BACKGROUND AND AIMS: N-acetyltransferase 2 (NAT2) is a drug-metabolizing enzyme, which is genetically variable in human populations. Polymorphisms in the NAT2 gene have been associated with drug efficacy and toxicity as well as disease susceptibility. Recently, an association of NAT2 gene variation with risk of type 2 diabetes mellitus (T2DM) has been suggested. This is the first study performed in a population from Bosnia and Herzegovina (BH) in which the frequency of two common NAT2 polymorphisms, 341T>C (NAT2*5) and 590G>A (NAT2*6) was determined in diabetic patients. METHODS: The frequency of the NAT2*5 (341T>C) and NAT2*6 (590G>A) polymorphisms was analyzed by employing TaqMan SNP Genotyping Assays (Applied Biosystems) in a group of 63 patients with T2DM and 79 nondiabetic subjects. RESULTS: Our data demonstrated that the frequencies of NAT2*5 (341T>C) and NAT2*6 (590G>A) polymorphisms in BH population were in line with the Caucasians genotype data. The NAT2*5 and NAT2*6 alleles were in high linkage disequilibrium (D' = 0.969). Strinkingly, there was a significant difference in genotype frequencies for NAT2*5 ($p < 0.05$) and NAT2*6 ($p < 0.001$) polymorphisms between diabetic and nondiabetic subjects. NAT2*5 polymorphism was associated with 2.4-fold increased risk for developing T2DM (adjusted OR = 2.40, 95% CI = 1.10-5.25, p = 0.028). On the contrary, NAT2*6 variant significantly decreased by 5-fold susceptibility to the disease (adjusted OR = 0.20, 95% CI = 0.09-0.43, $p < 0.001$). CONCLUSIONS: Our data demonstrated that NAT2 genetic variation appeared to be an important risk factor in development of T2DM.

Švraka E, Loga S, Brown I. Family quality of life: adult school children with intellectual disabilities in Bosnia and Herzegovina. J Intellect Disabil Res. 2011 Jun 13. doi: 10.1111/j.1365-2788.2011.01434.x. [Epub ahead of print]

Faculty of Health Studies, University of Sarajevo, Sarajevo, Bosnia and Herzegovina; Academy of Sciences and Arts of Bosnia and Herzegovina, Sarajevo, Bosnia and Herzegovina; Faculty of Social Work, University of Toronto, Toronto, Ontario, Canada.

Aims: This study endeavours to provide initial data on quality of life for families with adult children who have intellectual disabilities (ID) in the Canton of Sarajevo. Methods: The principal measure used was the Family Quality of life Survey 2006 - main caregivers of people with intellectual or developmental disabilities. The sample consisted of the main caregivers of 35 families with adult school children with ID who attended classes in a specially adapted programme in the Center of Vladimir Nazor and in the Vocational Secondary School in Sarajevo. Of the 35 participants, 21 were male and 14 female. Students with disabilities ranged from 19 to 32 years old (mean 21.45). Results: Consistent with previous research, the nine domains measured by the scale were all rated high for Importance. Opportunities were considered to be particularly low for Financial Well-Being and Support from Others. These domains were also rated lowest for Attainment and Satisfaction. Initiative was relatively high across all domains, and Stability (the degree to which things were seen as likely to improve or decline) varied slightly across domains but the means indicated that things are expected to stay almost the same or improve slightly. Conclusions: This research provides initial data for family quality of life in Bosnia and Herzegovina. It also provides suggestions for improving quality of life for families that have one or more members with ID. The results should also contribute to rejecting stereotypes and promoting inclusion of children with ID as well as the rights of their families.

Zalihić D, Zalihić A. Comparison of the results of surgical and non-surgical treatment of combat urogenital injuries in Bosnia war 1992-1995. Coll Antropol. 2011 Mar;35(1):227-33.

University of Mostar, School of Medicine, Mostar, Bosnia and Herzegovina.

Goal was to compare the results of surgical and non-surgical treatments of combat injuries of genitourinary system and to compare our data with data collected in the recent studies. The study was designed as a retrospective review of data collected in prospective databases. The data extracted from inpatients' medical records included demographics, mechanisms and type of injury, distribution of the lesions, clinical presentation features, applied diagnostic studies, treatment modalities, types of complication and results of treatment. Among 4.125 patients treated in the Mostar War Hospital, 111 had injury of genitourinary tract: 62 underwent a surgical and 49 non-surgical treatment. Mortality among operated patients was 16 (26%). Complications were noted in 47 patients (42%); in 33

(70%) were manifested as early complications, and 14 (30) as delayed ones (p = 0.006). Among the surgically treated patients, 40 (36%) had some complication, in comparison to 8 (7.2%) patients with complications among non-surgically treated patients; which represent a statistically significant difference (p < 0.05). In this study, there was a surprisingly high number of non-surgically treated patients, and this sub-group of UGT trauma patients had in some ways the superior treatment results in comparison with surgically treated patients. Conservatively treated patients had lower rate of complications, no mortality, and no patients with permanent disability.

Zerem E. Comment on the article about the evaluation of transabdominal ultrasonography performed by a gastroenterologist in his office: why should not all clinicians use transabdominal ultrasonography on a routine basis? J Clin Gastroenterol. 2011 May-Jun;45(5):476-7.

No abstract available.

Zerem E, Imamović G, Mavija Z, Haračić B. Comments on the article about correlation between computerized tomography and surgery in acute pancreatitis. World J Gastroenterol. 2011 Jan 21;17(3):407-8.

We read with great interest the article by Vege et al published in issue 34 of World J Gastroenterol 2010. The article evaluates the ability of contrast-enhanced computerized tomography (CECT) to characterize the nature of peripancreatic collections found at surgery. The results of their study indicate that most of the peripancreatic collections seen on CECT in patients with severe acute pancreatitis who require operative intervention contain necrotic tissue and CECT has a limited role in differentiating various types of collections. However, there are some points that need to be addressed, including data about the stage of acute pancreatitis in which CECT was done and the time span between CECT examination and surgery

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Zerem E, Imamović G, Omerović S. What is the optimal treatment for pancreatic pseudocysts? Scand J Gastroenterol. 2011 Jun 30. [Epub ahead of print]

University Clinical Center Tuzla, Trnovac bb, Tuzla, Bosnia and Herzegovina.

No abstract available.

Zerem E, Imamović G, Sušić A, Haračić B. Step-up approach to infected necrotising pancreatitis: a 20-year experience of percutaneous drainage in a single centre. Dig Liver Dis. 2011 Jun;43(6):478-83. Epub 2011 Apr 8.

University Clinical Center Tuzla, Tuzla, Bosnia and Herzegovina.

AIM: To evaluate the efficacy of step-up approach to infected necrotising pancreatitis. METHODS: Retrospective analysis of 86 patients treated by step-up approach from 1989 to 2009. Infection was confirmed by examination of aspirated material or by presence of free pancreatic gas at contrast-enhanced computed tomography. Conservative treatment was initially attempted in all patients; percutaneous catheter drainage was performed when conservative therapy failed; surgery was planned only if no clinical improvement was observed. Primary outcome was mortality. RESULTS: Fifteen patients (17.4%) were successfully treated with conservative treatment only. Percutaneous catheter drainage was performed in 69 (80.2%). Eight patients (9.3%) died, two at week 1 without drainage or surgery and six after percutaneous catheter drainage and surgery. Eleven patients were converted to surgery (12.8%). Organ failure occurred in 59/86 (68.6%) and multiorgan failure in 25/86 (29.1%). Median (interquartile ranges) hospital stay and catheter dwell times were 13 (9-47) and 15 (7-34) days, respectively. There were 2.61 catheter problems and 1.68 catheter changes per patient. CONCLUSIONS: The step-up approach is an effective and safe strategy for the treatment of infected necrotising pancreatitis. Percutaneous drainage can avert the need for surgery in the majority of patients.

Zerem E, Salkić N, Sušić A, Haračić B. Comments on the article about recurrence after surgical management of liver hydatid cyst. J Gastrointest Surg. 2011 Mar;15(3):536-7; author reply 538-9. Epub 2011 Jan 5.

No abstract available.

by Nerma Tanović

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Acknowledge. Anyone who contributed towards the study by making substantial contributions to conception, design, acquisition of data, or analysis and interpretation of data, or who was involved in

drafting the manuscript or revising it critically for important intellectual content, but who does not meet the criteria for authorship. List the source(s) of funding for the study and for the manuscript preparation in the acknowledgements section.

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Tables. Need to be submitted separate from the main text. The preferred software for tables is Microsoft Excel (save each table in a file with single worksheet). Only tables made with table tools in Microsoft Word are acceptable. For the paper version, type or print each table on a separate sheet of paper. Number tables consecutively in the order of their first citation in the text. Use Arabic numerals. Each table needs to have an explanatory title. Place the title above the table. Give each column a short or abbreviated heading. Also, visibly indicate the position of each table in the text, using its assigned numeral at the end of the sentence which is relevant to the table(s). Tables should be positioned in the text where the author feels is appropriate but the Editor reserves the right to re-organize the layout to suit the printing process. Authors need to place explanatory matter in footnotes, not in the heading. Explain in footnotes of the table all nonstandard abbreviations. For footnotes use the following symbols, in sequence: *, †, ‡, §, ||, ¶, **, ††, ‡‡. Identify statistical measures of variations, such as standard deviation and standard error of the arithmetic mean. *Be sure that each table is cited in the text.* If you use data from another published or unpublished source, obtain permission and acknowledge them fully.

Figures (illustrations: diagram, photograph, photomicrograph, radiograph, drawing, sketch, picture, outline, design, plan, map, chart, etc.). Need to be submitted separate from the main text. They need to be submitted as photographic quality digital prints or, exceptionally, as professionally drawn and photographed original illustrations. Figures should be in a digital format that will produce high quality images. Formats recommended include: JPEG, GIF, TIFF, Microsoft Word, Excel. Sending original photographs and slides is permissible when they cannot be digitized without professional help. In this case, send an explanation in the cover letter. Using Arabic numerals, number figures consecutively in the order of their first citation in the text. Also, visibly indicate the position of each figure in the text, using its assigned numeral in parentheses. Figures should be positioned in the text where the author feels is appropriate but the Editor reserves the right to re-organize the layout to suit the printing process.

Supply a legend for each figure. Titles and detailed explanations belong in the legends, however, not on the figures themselves. Figures should be made as self-explanatory as possible. Letters, numbers, and symbols on figures should therefore be clear and even throughout, and of sufficient size that when reduced for publication each item will still be legible. Photomicrographs should have internal scale markers. Symbols, arrows, or letters used in photomicrographs should contrast with the background. If photographs of people are used, either the subjects must not be identifiable or their pictures must be accompanied by written permission to use the photograph.

Legends for Figures need to be included in the main manuscript text file, on a separate page - immediately following the references. Type or print out legends using double spacing.

For each figure, the following information should be provided: figure number (in sequence, using Arabic numerals - i.e. Figure 1 or Fig. 1); title of the figure; all necessary explanations. When symbols, arrows, numbers, or letters are used to identify parts of the illustrations, identify and explain each one clearly in the legend.

Units of measurement

Measurements of length, height, weight, and volume should be reported in metric units (meter, kilogram, or liter) or their decimal multiples. Temperatures should be in degrees Celsius. Blood pressures should be in millimeters of mercury, unless other units are specifically required by the journal.

Abbreviation, Acronyms and Symbols

If possible for metric units use standard abbreviations. Non-standard abbreviations should be defined when first used in the text.

Sample references

Articles in journals

Standard journal article (*List the first six authors followed by et al.*):

Halpern SD, Ubel PA, Caplan AL. Solid-organ transplantation in HIV-infected patients. N Engl J Med. 2002;347(4):284-7.

More than six authors:

Rose ME, Huerbin MB, Melick J, Marion DW, Palmer AM, Schiding JK, et al. Regulation of interstitial excitatory amino acid concentrations after cortical contusion injury. Brain Res. 2002;935(1-2):40-6.

Organization as author:

Diabetes Prevention Program Research Group. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. Hypertension. 2002;40(5):679-86.

No author given:

21st century heart solution may have a sting in the tail. BMJ. 2002;325(7357):184.

Volume with supplement:

Geraud G, Spierings EL, Keywood C. Tolerability and safety of frovatriptan with short- and long-term use for treatment of migraine and in comparison with sumatriptan. Headache. 2002;42(Suppl 2):S93-9.

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Banit DM, Kaufer H, Hartford JM. Intraoperative frozen section analysis in revision total joint arthroplasty. Clin Orthop. 2002;(401):230-8.

Letters or abstracts:

Tor M, Turker H. International approaches to the prescription of long-term oxygen therapy [letter]. Eur Respir J. 2002;20(1):242. ; Lofwall MR, Strain EC, Brooner RK, Kindbom KA, Bigelow GE. Characteristics of older methadone maintenance (MM) patients [abstract]. Drug Alcohol Depend. 2002;66 Suppl 1:S105.

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Personal author(s):

Murray PR, Rosenthal KS, Kobayashi GS, Pfaller MA. Medical microbiology. 4th ed. St. Louis: Mosby; 2002.

Editor(s), compiler(s) as author:

Gilstrap LC 3rd, Cunningham FG, VanDorsten JP, editors. Operative obstetrics. 2nd ed. New York: McGraw-Hill; 2002.

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Royal Adelaide Hospital; University of Adelaide, Department of Clinical Nursing. Compendium of nursing research and practice development, 1999-2000. Adelaide (Australia): Adelaide University; 2001.

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Meltzer PS, Kallioniemi A, Trent JM. Chromosome alterations in human solid tumors. In: Vogelstein B, Kinzler KW, editors. The genetic basis of human cancer. New York: McGraw-Hill; 2002. p. 93-113.

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Borkowski MM. Infant sleep and feeding: a telephone survey of Hispanic Americans [dissertation]. Mount Pleasant (MI): Central Michigan University; 2002.

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Tynan T. Medical improvements lower homicide rate: study sees drop in assault rate. The Washington Post. 2002 Aug 12;Sect. A:2 (col. 4).

Dictionary and similar references:

Dorland's illustrated medical dictionary. 29th ed. Philadelphia: W.B. Saunders; 2000. Filamin; p. 675.

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CD-ROM:

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Abood S. Quality improvement initiative in nursing homes: the ANA acts in an advisory role. Am J Nurs [serial on the Internet]. 2002 Jun [cited 2002 Aug 12];102(6):[about 3 p.]. Available from: http://www.nursingworld.org/AJN/2002/june/Wawatch.htm

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Foley KM, Gelband H, editors. Improving palliative care for cancer [monograph on the Internet]. Washington: National Academy Press; 2001 [cited 2002 Jul 9]. Available from: http://www.nap.edu/books/0309074029/html/.

Homepage/Web site:

Cancer-Pain.org [homepage on the Internet]. New York: Association of Cancer Online Resources, Inc.; c2000-01 [updated 2002 May 16; cited 2002 Jul 9]. Available from: http://www.cancer-pain.org/.

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American Medical Association [homepage on the Internet]. Chicago: The Association; c1995-2002 [updated 2001 Aug 23; cited 2002 Aug 12]. AMA Office of Group Practice Liaison; [about 2 screens]. Available from: http://www.ama-assn.org/ama/pub/category/1736.html

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